REGULATION OF FRUCTOSE 1,6-DIPHOSPHATASE IN YEASTS

A THESIS

SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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DEPARTMENT OF BIOLOGY

ATLANTA, GEORGIA

AUGUST 1974

ACKNOWLEDGMENTS

The author wishes to thank members of the thesis committee for their interest throughout this work. He is especially indebted to Dr. Winfred Harris for his untiring efforts and helpful suggestions; to Dr. Roy Hunter, Jr. and Dr. Lafayette Frederick for their encouragement to enter the Ph.D. program; and to Mrs. L. Frederick for her understanding which has been invaluable throughout his graduate studies.

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CHAPTER I

INTRODUCTION

Traditional pathways of metabolism have been classified into two major categories, anabolic (biosynthetic) and catabolic (degradative). Procaryotic organisms, like bacteria, utilize glycolytic enzymes in their catabolic pathways when glucose is supplied as the carbon source. However, when C_3 or C_4 organic acids are supplied, the same basic set of glycolytic enzymes function in anabolic pathways. The term, amphibolic, implies pathways in which anabolic and catabolic functions are realized.

Studies of anabolic and catabolic regulation of gene expression and their ultimate effects on enzyme activity have been extensively verified in procaryotic organisms. The fundamental mechanisms of information flow from DNA directing enzyme biosynthesis in bacteria are virtually identical to those found in eucaryotic cells. However, certain features of the morphology of the genetic apparatus of eucaryotic cells are very different from their analogous structures in bacteria. These differences imply that the mechanisms regulating enzyme expression in procaryotes and eucaryotic cells may also be significantly different.

It is generally thought that all eucaryotes are essentially the same in the regulation of gene expression. However, recent evidence is accumulating that fungal nuclei lack histones. Hence, regulation of gene expression and its related effects on enzyme

expression in fungi may be different from that in other eucaryotes.

Yeasts offer a number of potential advantages in studies dealing with the regulation of proteins. Of paramount importance is the fact that these organisms, i.e., Saccharomyces cerevisiae, are intermediate in the complexity of their protein-synthesizing cell machinery having characteristics of bacteria as well as mammals.

Saccharomyces cerevisiae has a well defined sexual phase in its life cycle and its nucleus contains several linkage groups. The amino acid-activating enzymes, tRNA, and ribonucleoprotein particles have been isolated and extensively studied in yeasts. Further, a large number of highly purified and well-characterized proteins, many inducible, have been isolated, thus offering a wide range of choice for the study of the regulation or biosynthesis of a particular protein in vivo as well as in cell-free systems.

In the direction of gluconeogenesis, fructose 1,6-diphosphatase (FDPase) is essential for the formation of fructose 6-phosphate from fructose 1,6-diphosphate (FDP). Specifically, FDPase hydrolyzes the 1-phosphate group of FDP to yield fructose 6-phosphate. Fructose 1,6-diphosphatase has been found to be adaptable in yeasts being inducible on non-fermentable carbon sources and inactivated in the presence of glucose. Glucose has been found to prevent the formation of many inducible enzymes in yeasts. The effect of glucose often is indistinguishable from repression, the end-product apparently being an intermediate in catabolism (i.e., a catabolite), whose intracellular concentration rises when a rapidly utilizable source of catabolism, such as glucose, is supplied. In this regard, the "glucose effect" is

often referred to as "catabolite repression".

The "glucose effect" can generally be exerted by any carbon source which can support more rapid growth than the inducer. On the other hand, glucose fails to exert its characteristic effects when its metabolism is slowed by permeability mutations. Thus, ample evidence exists showing that the effector of catabolite repression is not glucose itself or some other sugar supplied as a substrate since catabolite repression requires the rapid metabolism of the substrate. It may well be visualized that a type of transient repression of inducible enzymes occurs in all cell types that encounter a new carbon source in their growth media. The newly added compound need not be metabolized in order to repress. Simply, the passage of the compound through the cell membrane is responsible for the repression. It is therefore possible that both control mechanisms, catabolite repression and transient repression, operate through a common effector molecule. However, in the final analysis, it is more probable that different catabolites are responsible for the repression of different enzymes and enzyme systems.

Transcriptional control of inducible enzyme synthesis has been demonstrated for a variety of bacterial systems. More recent works, however, have indicated that in some cases, translation also seems to be affected by specific regulatory processes. The use of DNA-RNA hybridization techniques on the one hand, and of specific metabolic inhibitors plus pulse-induction on the other, has allowed one to estimate the synthesis and stability of specific messengers.

Through the use of inhibitors of protein synthesis during

induction of enzymes in fungi, models coupling the extent of mRNA accumulation to its stability, determined by translation have been found valid. However, it must be kept in mind that most eucaryotes form considerable amounts of RNA of various species which never leaves the nucleus. The accumulation of various RNA's under the influence of inhibitors makes the interpretation of data not an easy task.

Further, in eucaryotic cells, the genes specifying a given multienzyme system are frequently scattered among several linkage groups. Hence, the genetic mechanisms of induction and repression, whether or not coordinate, of multienzyme systems in lower and higher eucaryotes are thus rather different from those in procaryotes and are still largely unknown.

Since the energetics of glycolysis have been extensively developed, it is obvious that a simple reversal of this process does not adequately account for gluconeogenesis in yeast. For this reason, the present investigation focuses on one gluconeogenic enzyme, FDPase, as a possible site for the control of this process. The experimental work described investigates the level(s) of action of the regulatory mechanism(s) involved in the induction and glucose-induced "inactivation/repression" of FDPase in cells of Saccharomyces cerevisiae. In addition, the effects of inhibitors of RNA and protein synthesis on FDPase induction and repression was studied. It is experimentally difficult, if not indeed impossible, to prevent specifically either RNA or protein synthesis while not affecting the other process. The use of inhibitors to stop selective synthesis of different macromolecules leads inevitably to various uncertainties: precisely, how do

they work; is only one type of inhibitory process elicited; and, if more than one process is inhibited, were these processes associated before the presence of the inhibitor or dissociated after adding the inhibitor? These factors must always be considered before and during the interpretation of experimental results.

CHAPTER II

REVIEW OF LITERATURE

The presence in mammalian liver of a specific phosphatase which catalyzes the hydrolysis of fructose 1,6-diphosphate (FDP) was first reported by Gomori (1943). Fructose 1,6-diphosphatase (D-fructose 1,6-diphosphate-1-phosphohydrolase, EC 3.1.3.11) (FDPase), an enzyme of the gluconeogenic pathway, has been found to be adaptive in yeasts.

In the direction of gluconeogenesis, FDPase is essential for the formation of fructose 6-phosphate from FDP. Specifically, according to Pogell and McGilvery (1952), FDPase hydrolyzes the 1-phosphate group of FDP to yield fructose 6-phosphate. Hers and Kusada (1953) have partially elucidated the role of this enzyme during the metabolism of fructose in mammalian liver. Mokrasch, Davidson and McGilvery (1956) have identified a specific role of FDPase in gluconeogenesis by noting that conditions which favored gluconeogenesis resulted in increased levels of the enzyme in rabbit liver. Fraenkel and Horecker (1965) have provided additional support for the contention that FDPase has a gluconeogenic function. In their work, bacterial strains deficient in a specific FDPase were not capable of growth on compounds such as glycerol, acetate and succinate.

Fructose 1,6-diphosphatase activity has been described in a wide variety of organisms; McGilvery and Pogell (1961) described its activity in animals, Racker and Schroeder (1958) in plants, and Rosen (1966) in microbial organisms. All of these organisms share a number of

similar properties in their FDPase: They generally require an alkaline pH for optimal activity; EDTA stimulates activity in the neutral pH range; a divalent cation, magnesium or manganese; and they have a low Km for substrate and are inhibited by low concentrations of 5'-AMP.

Bonsignore, Mangiarotti, Mangiarotti, DeFlora and Pontremoli (1963) found that purified FDPase from mammalian liver catalyzed the hydrolysis of FDP and sedoheptulose diphosphate (SDP). Rosen, Rosen and Horecker (1965b) found that the purified enzyme from one genus of yeast, Candida utilis, is inactive with SDP. Generally substrates other than FDP and SDP are hydrolyzed very slowly, if at all.

Pontremoli et al. (1971) have observed that when the "neutral" FDPase from mammalian liver was digested with papain, the enzyme was converted to a form with an alkaline pH optimum. Further changes in catalytic activity noted were changes in the ratio of activities toward FDP and SDP, accompanied by desensitization to AMP inhibition. More recently, Pontremoli et al. (1973) noted that subtilisn digestion of rabbit liver FDPase resulted in the removal of a peptide near the NH_2-terminus containing a single tryptophanerresidue. The authors concluded that the loss of a tryptophane-containing peptide is responsible for the conversion of the native "neutral" FDPase to an "alkaline" form. Allen and Blair (1972) have isolated a potent activator of rabbit liver FDPase in vitro tentatively identified as a phospholipid, diphosphoinositide. Since this compound turns over rapidly and is present in vivo at low concentration, the authors suggest that it may play a role in in vivo regulation of FDPase in the liver.

It is now widely accepted that both glycolysis and gluconeogenesis are in part largely regulated at the level of FDP formation and hydrolysis, respectively. During glycolysis, Ramiah et al. (1964) illustrated that the activity of phosphofructokinase was inhibited by adenosine triphosphate (ATP) and citrate. However, this inhibition was reversed by AMP. Thus, a rise in the concentration of intracellular AMP should enhance PFKase activity and prevent the breakdown of FDP to fructose 6-phosphate, thereby, promoting glycolysis. On the other hand, a decrease in the concentration of AMP should release FDPase from inhibition and allow metabolism to proceed in the direction of gluconeogenesis. Taketa and Pogell (1965) and Gancedo, Salas, Giner and Sols (1965) have indicated that sensitivity to AMP inhibition is a property of FDPase from many biological sources.

The homeostatic control of blood sugar depends in part on the regulation of gluconeogenic enzymes. Weber et al. (1965) focused on one-way reactions catalyzed by four gluconeogenic enzymes in mammalian liver, glucose 6-phosphatase, FDPase, PEP carboxykinase and pyruvate carboxylase. Evidence is presented by these authors which indicates that the genic expression of these key gluconeogenic enzymes is regulated by the action of glucocorticoid hormones as inducers and insulin as a suppressor of their biosynthesis.

Stancel and Deal (1969) have reported that ATP induces rapid but subtle conformational changes when bound to glyceraldehyde 3-phosphate dehydrogenase isolated from yeasts. The conformational changes increase the rate of inactivation of this enzyme by chymotrypsin and neutral pH but dissociation occurred slowly. This enzyme is important

in yeast glycolytic-gluconeogenic schemes and the effects of ATP offer another control mechanism of metabolic processes other than induction-repression and activation-inhibition.

Bianchetti and Satirana (1967) have described a nonphotosynthetic FDPase in higher plants which also appears to be regulated allosterically by 5'-AMP. Their work with wheat embryo tissues and the changes in levels of this enzyme in response to various physiological conditions support a gluconeogenic role for the enzyme. Smillie (1960, 1964) has provided evidence for the function of an alkaline FDPase in higher plants and Euglena. The FDPase was associated with photosynthetic tissue being localized in the chloroplasts and was absent in non-photosynthetic tissues and bleached algae. Preiss et al. (1967) have demonstrated the localization of an alkaline FDPase in the chloroplast of spinach leaves.

Fossit and Bernstein (1963) purified a bacterial FDPase from extracts of Pseudomonas saccharophila and Sapico et al. (1968) reported FDPase in Aerobacter aerogenes; in both cases thesspecificity of the enzyme and the stoichiometry of the reaction were established. Mukadda and Bell (1969) described an FDPase in a species of gram-negative Acinetobacter which was inhibited by ATP and citrate, but not by AMP. The authors suggested that the role of this enzyme in this organism may be catabolic rather than anabolic. Rosen (1966) purified FDPase from the slime mold, Polysphondylium palidium, which hydrolyzed both FDP and SDP. However, this slime mold enzyme was not inhibited by AMP at any pH and the authors could not relate its activity to catabolic or anabolic processes occurring during various stages of differentiation.

Hence, the enzyme in this organism appears to be constitutive.

In microorganisms, the synthesis of several enzymes is repressed by adding glucose to the medium. Witt et al. (1966) have reported that malate dehydrogenase from a strain of Saccharomyces cerevisiae is repressed by glucose with apparent inactivation of the enzyme. Yeast grown in the presence of glucose have low levels of FDPase; however, when these organisms are grown on non-fermentable carbon sources, i.e., lactate, acetate, glycerol, and ethanol, Rosen et al. (1965a) have reported an increase in the specific activity of FDPase. The term "catabolite repression" has been introduced by Magasanik (1961) to describe enzyme repression by glucose.

Nakada and Magasanik (1964) have provided results from a study on the regulatory effects of induction and catabolite repression on the synthesis of messenger RNA (mRNA) coding for beta-galactosidase in Escherichia coli. Their work led to the conclusion that the inducer increases and that catabolite repression decreases the rate of synthesis of mRNA specific for beta-galactosidase. However, induction of beta-galactosidase in E. coli can occur in some cells apparently without the formation of the galactoside permease necessary to initiate accumulation of the inducer (Maloney and Rotman, 1973). Further, the control mechanisms involved in the regulation of this enzyme in E. coli involve a type of "transient repression" occurring at the cell membrane during growth on glucose (Tyler and Magasanik, 1969). This type of repression does not require the lac-I-gene product and is elicited by glucose analogues that are phosphorylated

but not further catabolized by the cell. Hence, transient repression and catabolite repression prevent the synthesis of beta-galactosidase-specific RNA in E. coli. Further, the work of Wijk et al. (1969) concerned with alpha-glucosidase synthesis in S. carlbergensis indicated that glucose repression of the synthesis of this enzyme may in addition operate at the translational level. Hauge et al. (1961) working with beta-glucosidase in yeast have suggested the possibility that glucose exerts its repressing effect during protein synthesis at the ribosomal level.

In a study of the derepression of a low Km glucose uptake system (system II) in Neurospora, Schneider and Wiley (1971) noted that glucose prevented the accumulation of mRNA specific for system II even in the presence of cycloheximide. The conclusion reached was that at least for this system, the site of catabolite repression was at the level of transcription. Repression of enzyme synthesis at the transcriptive level has also been noted for an inducible kynureninase in Neurospora by Turner et al. (1970). Horowitz et al. (1970) reported repression of tyrosinase in Neurospora via an unstable repressor acting at transcription, and Nebert and Gelboin (1970) indicated that aryl hydrocarbon hydroxylase induction in foetal hamster cells in culture apparently occurs at the level of transcription. However, Tomkins et al. (1969), while observing induction and repression of mammalian tyrosine aminotransferase proposed that these control mechanisms in eucaryotes are regulated by a labile repressor; that the repressor inhibits translation and promotes mRNA breakdown rather than repression at the level of transcription.

Cazzulo et al. (1968) have investigated the levels of pyruvate carboxylase (PC) and phosphoenol pyruvate carboxylase (PEPC) in wild-type S. cerevisiae in the presence and absence of glucose. The authors suggested that these two enzymes are subject to different control mechanisms. The activity of PC is regulated through activation and inhibition by metabolites while PEPC is regulated through changes in the rate of enzyme synthesis. Lusis and Becker (1973) reported that glucose represses the induction of cellobiase by cellulose in the thermophilic fungus, Chaetomium thermophile. The inactivation by glucose was assumed to be irreversible because cycloheximide prevented de novo synthesis of the enzyme during induction.

Ferguson et al. (1967) noted that malate dehydrogenase activity in yeast was apparently inactivated following exposure to glucose. The inactivation was found to be prevented by the inhibition of protein synthesis with cycloheximide. The specific FDPase from S. cerevisiae has been found also to undergo "inactivation/repression" after the addition of glucose to the culture medium, as noted by Harris and Ferguson (1967). Gancedo (1971) reported that glucose addition to a derepressed culture of S. cerevisiae led to a rapid loss of the measurable activity of FDPase. However, Gancedo's experiments with cycloheximide indicated thatthe inactivation by glucose did not require protein synthesis.

Since FDPase catalyzes a critical and essentially irreversible step in gluconeogenesis, its regulation in vivo is of particular interest.

CHAPTER III

MATERIALS AND METHODS

Growth of Cultures

The strains of Saccharomyces cerevisiae used in this work were Y203 (a-adenine) and Y207 (alpha-adenine, histidine). These strains were provided by Dr. H. O. Halvorson, Department of Bacteriology, University of Wisconsin, Madison, WI. Two growth media were routinely used; an enriched medium consisted of 1.0% yeast extract-2.0% bacto-peptone (YEP) and 1.0% glucose as the carbon source. The minimal medium used was that described by Polakis and Bartley (1965). Both media were supplemented with adenine (20 mg/l) or adenine and histidine (20 mg/l) as required by the above strains.

Routinely, when large quantities of cells were desired, an initial loop inoculum was taken from an agar slant and inoculated into a 500 ml Erlenmeyer flask containing 100 ml of YEP and 1.0% glucose and shaken at 28 C for 24 hr. Cells were harvested by centrifugation, washed in 0.9% NaCl and resuspended for an additional 24 hr in a 2.8 l Erlenmeyer flask containing 450 ml of an identical medium. From a 1.0 g inoculum, 10.0-12 g (wet weight) of cells could be grown in 24 hr of incubation. These cells generally had high levels of FDPase. To obtain cells with repressed levels of FDPase, they were oollected by centrifugation, washed extensively in 0.9% NaCl and transferred to 450 ml of minimal medium in a 2.8 l flask containing an appropriate carbon source. In all experiments to be described, the initial carbon

source in minimal medium (after transfer from YEP) was 2.0% glucose in which the cells remained while shaking for 2-4 hr.

In most experiments, to obtain an initial induced enzyme level, cells were harvested from the minimal and 2.0% glucose medium, washed extensively, and transferred to a 2.8 l flask containing 450 ml of minimal medium, 1.0% acetate, adenine or adenine and histidine. The cells were incubated while shaking for 4-8 hr. Sterile growth conditions and techniques were employed throughout all experiments.

Chemicals

The following products were purchased from Sigma Chemical Company, St. Louis, MO: Pyruvic acid (Na salt), DL-histidine, 5-methyl tryptophane, 2-deoxy-D-glucose, 5'-adenosine monophosphate, cyclic 3', 5'-adenosine monophosphate, glucose 6-phosphate (monosodium salt), D(-)-3-phosphoglyceric acid (Na salt), phosphoenol pyruvate (Trisodium salt), fructose 6-phosphate (Na salt), and DL-alpha glycerophosphate (disodium salt).

The following were purchased from Calbiochem, San Diego, CA: Triphosphopyridine nucleotide (Na salt - A grade), phosphoglucose isomerase (yeast - A grade - 3768 I.U./ml), adenine, cycloheximide (Actidione), D-fructose, fructose 1,6-diphosphate (Na salt), and glucose 6-phosphate dehydrogenase (300 units/mg prot).

The J. T. Baker Chemical Co., Phillipsburg, NJ supplied sodium acetate and dextrose and Pfanstiehl Laboratories, Waukegan, IL supplied the D-mannose.

Preparation of Extracts

Two methods were utilized to prepare crude extracts depending

upon whether the collected samples were in 1.0 g (wet wt) quantities or 0.5 g quantities. In the former, cells were harvested by centrifugation, washed in 0.9% NaCl and resuspended in 3.0 ml of a 0.005M Tris-HCl buffer stabilized with 0.001M cysteine. Before use, the pH of the grinding buffer was adjusted to 7.5 with 1N NaOH. The suspension was added to a screw cap tube containing 3.0 g of chilled glass beads (Glasperlen - 0.45-0.50 mm). The cells were shaken for 90 sec in a CO_2 chilled Bronwill MSK Mechanical Cell Homogenizer at 4,000 rpm. After breakage, any remaining residue in the grinding tubes was removed with 0.5 ml of the grinding buffer.

The broken cells were centrifuged at 15,000 x G for 15 min in a refrigerated IEC High-Speed Centrifuge Model B-20. The precipitate was discarded and the supernatant collected and stored at -20 C until needed. The crude extracts were used in all enzyme assays and protein determinations. In the latter, one-half gram samples of cells were harvested by centrifugation, washed, and collected by filtration using Millipore filter disks (1.2 microns) under vacuum. The samples were ground in a cold mortar and pestle with three times their wet weight of alumina (305 - Aluminum Company of America, Alcoa, TN). The final volume of the Tris-HCl buffer was 3.0 ml. The total grinding time was 4 min. divided into 1 min intervals. The time between grinding intervals was used for mixing the alumina and buffer with the cells. The crude extracts were collected as mentioned for 1.0 g samples and used for all enzyme assays and protein determinations.

Measurement of Enzyme Activities

The FDPase assay system used was that devised by Gancedo, Salas,

Giner, and Sols (1965). The enzyme was assayed spectrophotometrically by measuring the reduction of TPN in a coupled system at pH 7.5 in 0.05M Tris-HCl buffer using a Beckman DBG-T recording spectrophotometer set at 340 mμ. Specific activity is expressed as μM TPNH formed/min/mg prot. Protein concentrations in the crude extracts were determined by the Biuret method.

CHAPTER IV

EXPERIMENTAL RESULTS

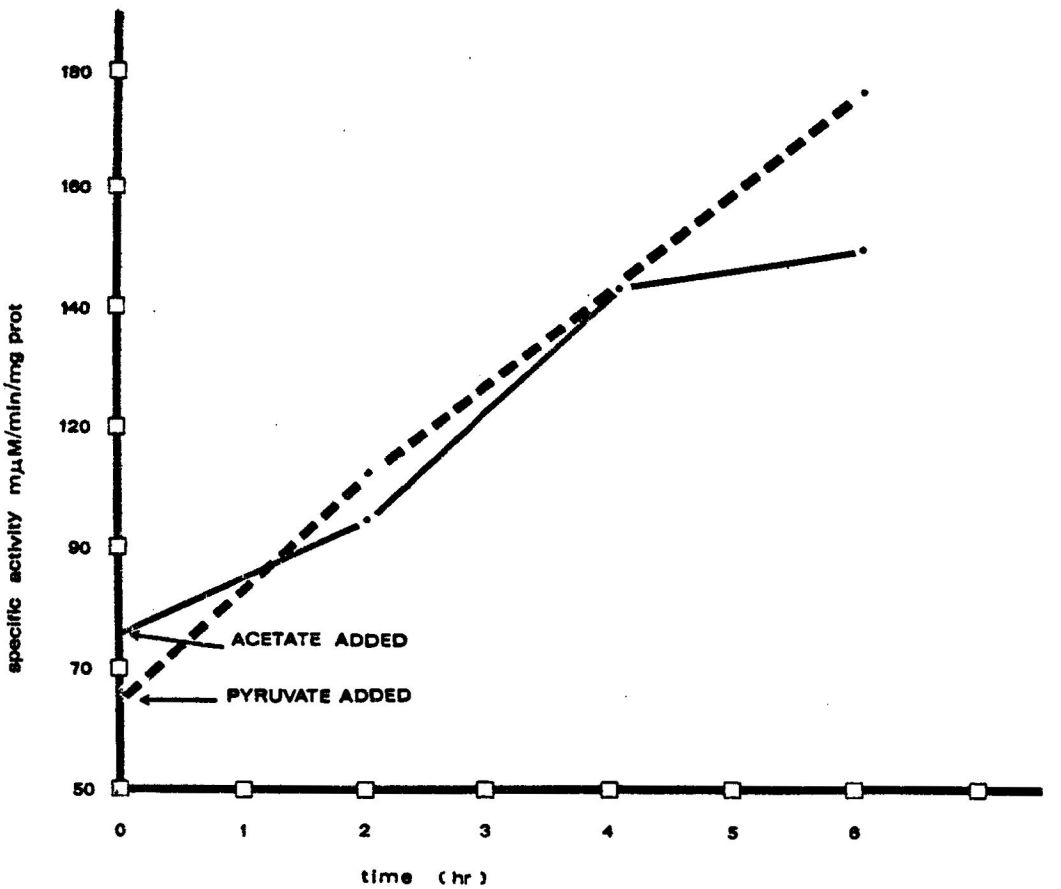
Induction of FDPase by Acetate and Pyruvate

The term, induction, is used in this work to indicate an increase in the rate of synthesis of an enzyme after the addition of an inducer. The term, derepression, is used interchangeably with induction in view of the fact that the molecular mechanism(s) responsible for the increase in specific activity cannot be identified at this time.

Illustrated in Fig. 1 are the results of induction (derepression) of FDPase in strain Y203 in minimal media with the addition of either acetate (1.0%) or pyruvate (1.0%) as sources of carbon. The time course of induction follows previous growth for 2 hr in minimal media containing 2.0% glucose. The activity of the enzyme after 2 hr growth in glucose is shown at time=0. Aliquots from the induction media were taken for assays at 2, 4, and 6 hr after the addition of carbon sources.

The data indicate that there is a time-dependent increase in specific activity of FDPase in each carbon source. The difference in specific activity shown at the end of the incubation period (6 hr) may not be significant. It should be mentioned, although not shown in the illustration, that after 12 hr of growth in induction media, repeatedly the specific activity of acetate-induced cells was higher than those induced in pyruvate. However, again the difference in specific activity appeared to be insignificant. It should be further stated that the rate of derepression of FDPase in each carbon source varied slightly from

Fig. 1. Induction of FDPase in strain Y203 in two different carbon sources: acetate (1.0%) (——— · ———) and pyruvate (1.0%) (-- · --) were added at zero time.


specific activity mμM/min/mg prot
180
160
140
120
90
70
50
ACETATE ADDED
PYRUVATE ADDED
0
1
2
3
4
5
6
time (hr)

preparation to preparation.

Figure 2 shows the time course of derepression of FDPase activity in strain Y207 with procedures being followed as depicted in Fig. 1. A marked difference in the rate of derepression occurs in this strain during growth on acetate and pyruvate. This difference may be due to the more rapid growth of Y207 in acetate than in pyruvate. Presumably, this growth rate difference may reflect a more rapid conversion of acetate into metabolites utilizable during gluconeogenic growth or the more rapid penetration of acetate into the cell. The decline in specific activity of FDPase after 2 hr of induction of acetate possibly reflects enzyme turnover due to such an accelerated initial synthetic level, coupled with a rapid rate of growth in this carbon source. Because of the erratic derepressive response of Y207 in acetate, no further induction experiments were performed with this strain. However, the data indicate that there is a strain difference in the response of Y203 and Y207 to the presence of acetate and pyruvate.

Inactivation/Repression by Glucose

The terms, "inactivation/repression", are used in this work functionally, as described by Ferguson et al. (1967), in which specific activity of an induced enzyme decreases after the addition of repressor.

In an effort to ascertain the effects of glucose on inactivation/repression of FDPase, the experiments illustrated in Figs. 3 and 4 were performed. Y203 were grown under repressive conditions (Fig. 3), then incubated for 12 hr in minimal media with either acetate or pyruvate as a carbon source. Aliquots were collected at 12 hr and the remaining cells were transferred to minimal media and 2.0% glucose for 2 hr.

Fig. 2. Induction of FDPase in strain Y207 in two different carbon sources: acetate (1.0%) (—— · ——) and pyruvate (1.0%) (-- · --) were added at zero time.

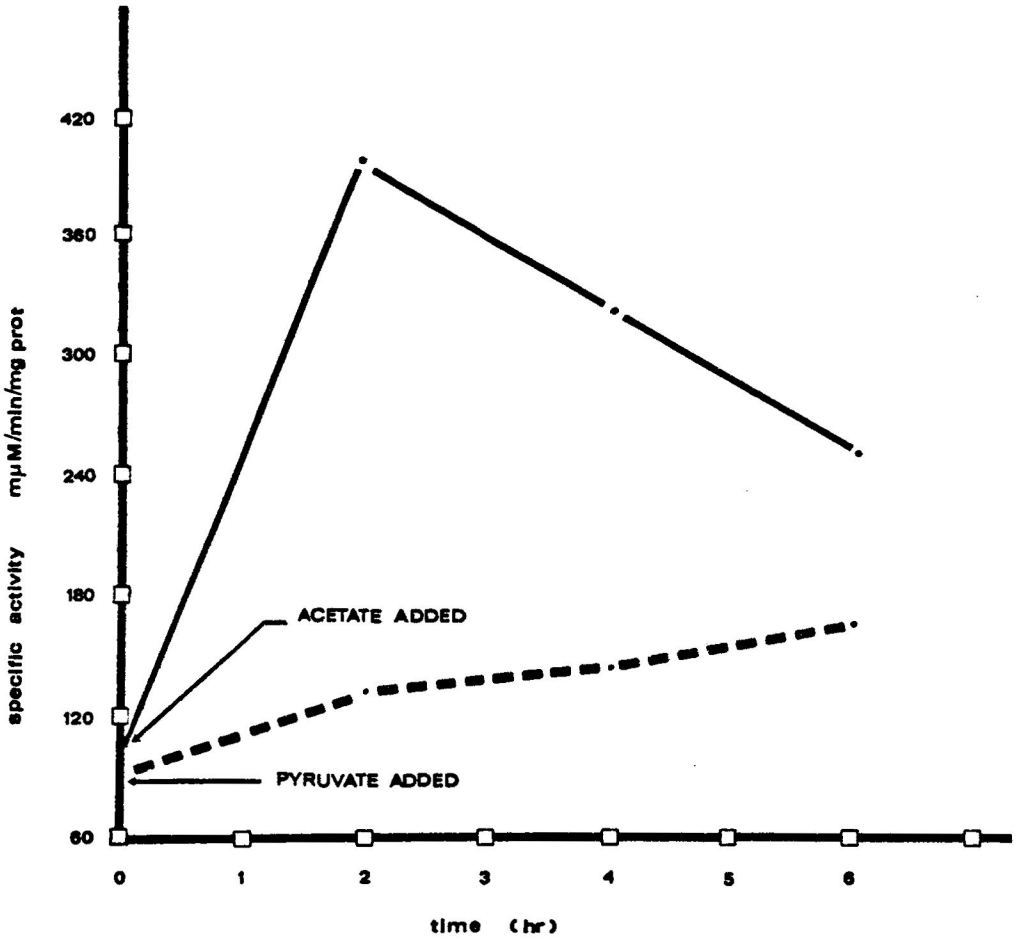

420
360
300
240
180
120
60
specific activity mμM/min/mg prot
ACETATE ADDED
PYRUVATE ADDED
0
1
2
3
4
5
6
time (hr)

Fig. 3. Inactivation/repression of FDPase by glucose in strain Y203. Cells were grown initially (0-time activity) for 2 hr in glucose (2.0%), then incubated in acetate (1.0%) (—— • ——) or pyruvate (1.0%) (-- • --)for 12 hr followed by additional growth for 2 hr in 2.0% glucose.

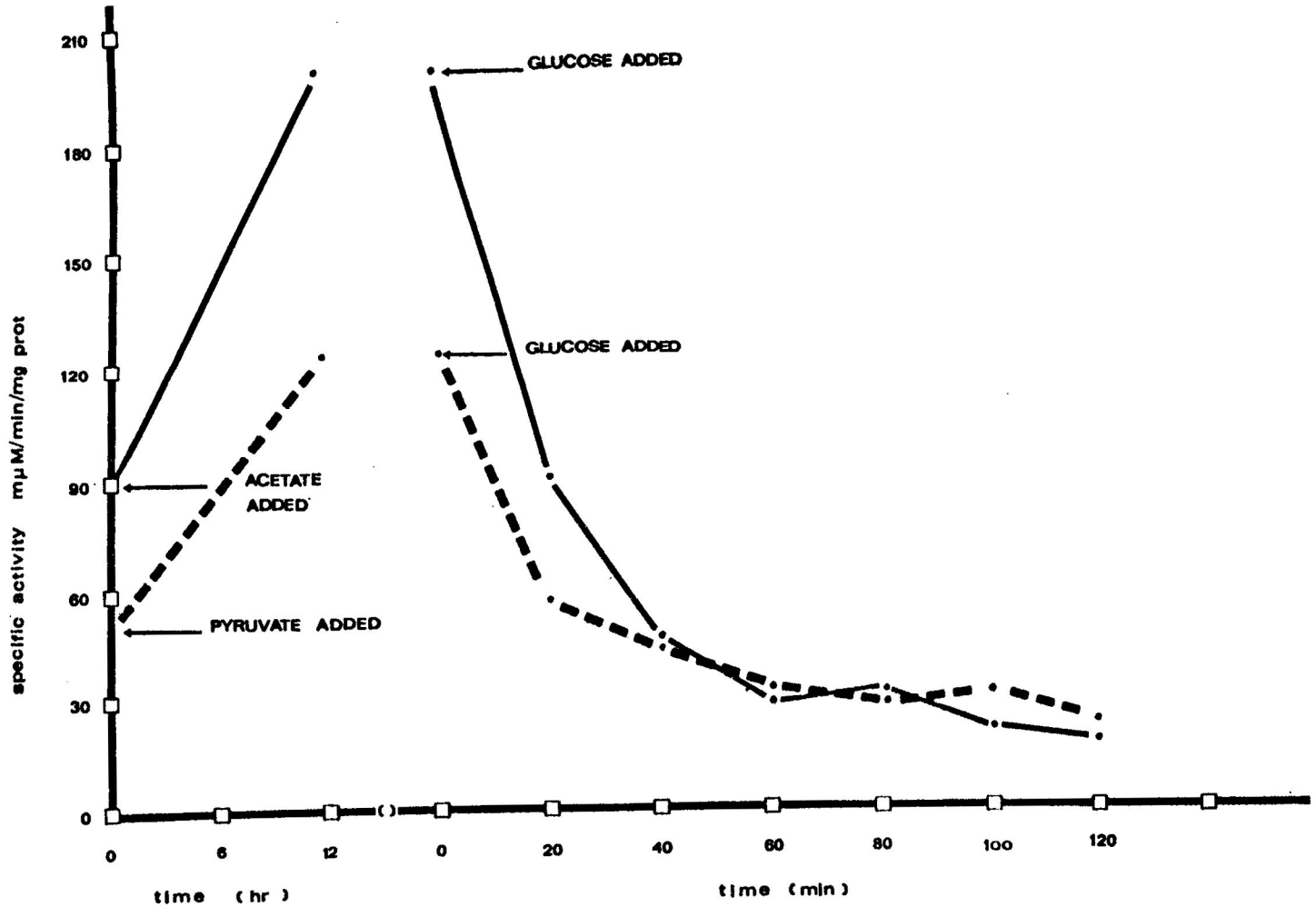
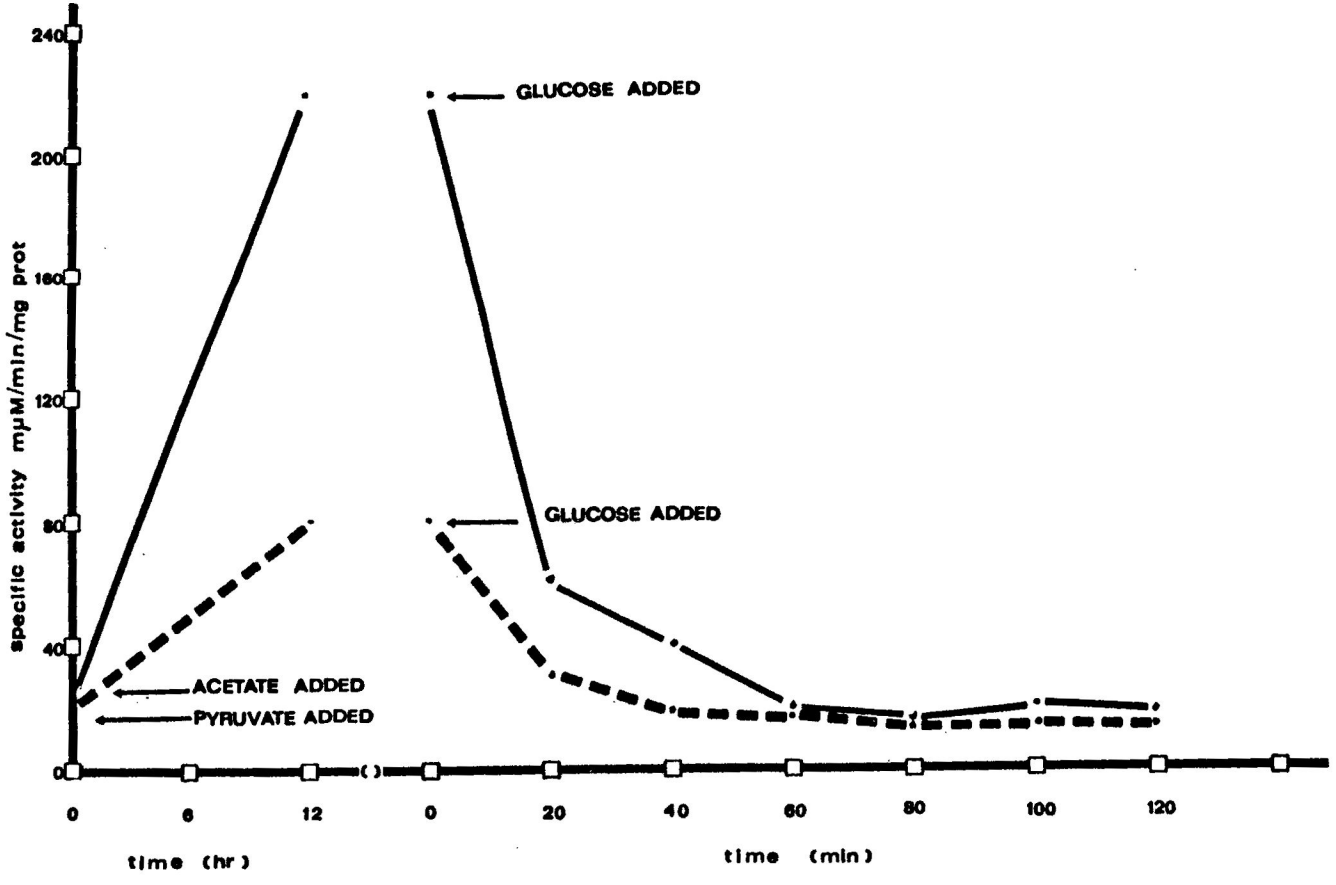

specific activity mμM/min/mg prot
210
180
150
120
90
60
30
0
GLUCOSE ADDED
GLUCOSE ADDED
ACETATE ADDED
PYRUVATE ADDED
0
6
12
0
20
40
60
80
100
120
time (hr)
time (min)

Fig. 4. Inactivation/repression of FDPase by glucose in strain Y207. Cells were grown initially (0-time activity) for 2 hr in glucose (2.0%), then incubated in acetate (1.0%) (——— · ———) or pyruvate (1.0%) (-- · --) for 12 hr followed by additional growth for 2 hr in 2.0% glucose.


specific activity mμM/min/mg prot
240
200
160
120
80
40
0
GLUCOSE ADDED
GLUCOSE ADDED
ACETATE ADDED
PYRUVATE ADDED
0
6
12
0
20
40
60
80
100
120
time (hr)
time (min)

Samples were harvested at the indicated time intervals.

The results in Fig. 3 illustrate that the decline in specific activity of FDPase or the glucose inactivation/repression is similar after derepression in acetate and pyruvate. The rationale for lengthening the induction period to 12 hr was to insure that the cultures would be in the fully-induced state before the addition of glucose. It should be noted that the declines in specific activities are much more rapid than the corresponding acetate or pyruvate-mediated increases (see Fig. 1). The activities of the enzyme return to the pre-induction levels within 20 min and continue to decline for the duration of the experiment (120 min). It appears that rapid chilling and storage under refrigeration of the yeast samples stabilizes the FDPase activity at the level present before chilling.

Figuee 4 shows the results of the same experiment with Y207. It is apparent that strain Y207 doesn't utilize pyruvate during induction (12 hr of incubation) nearly as effective as it does acetate. However, within 20 min, the declines of FDPase specific activities approach the pre-induction levels which here are apparently very close to the basal enzyme level. It should be noted that the 12 hr-induced level in acetate for Y207 is very close to the 12 hr-induced level in acetate seen for Y203 (Fig. 3).

Effects of Actinomycin D on the Derepression of FDPase

The antibiotic, actinomycin D, can inhibit the induced synthesis of a variety of enzymes in many organisms. Observations on the effects of this antibiotic on the induction of FDPase in Y203 are depicted in Figs. 5a, 5b, and 5c, respectively. Cells previously grown under

Fig. 5a. The effects of actinomycin D on the derepression of FDPase activity in acetate-induced cells of strain Y203. Zero-time activity represents 4 hr incubation in minimal media alone or with actinomycin D. Acetate (1.0%) was added to both media at 0-time. Control derepression (——— · ———), derepression in the presence of actinomycin D (80 μg/ml) (-- · --).

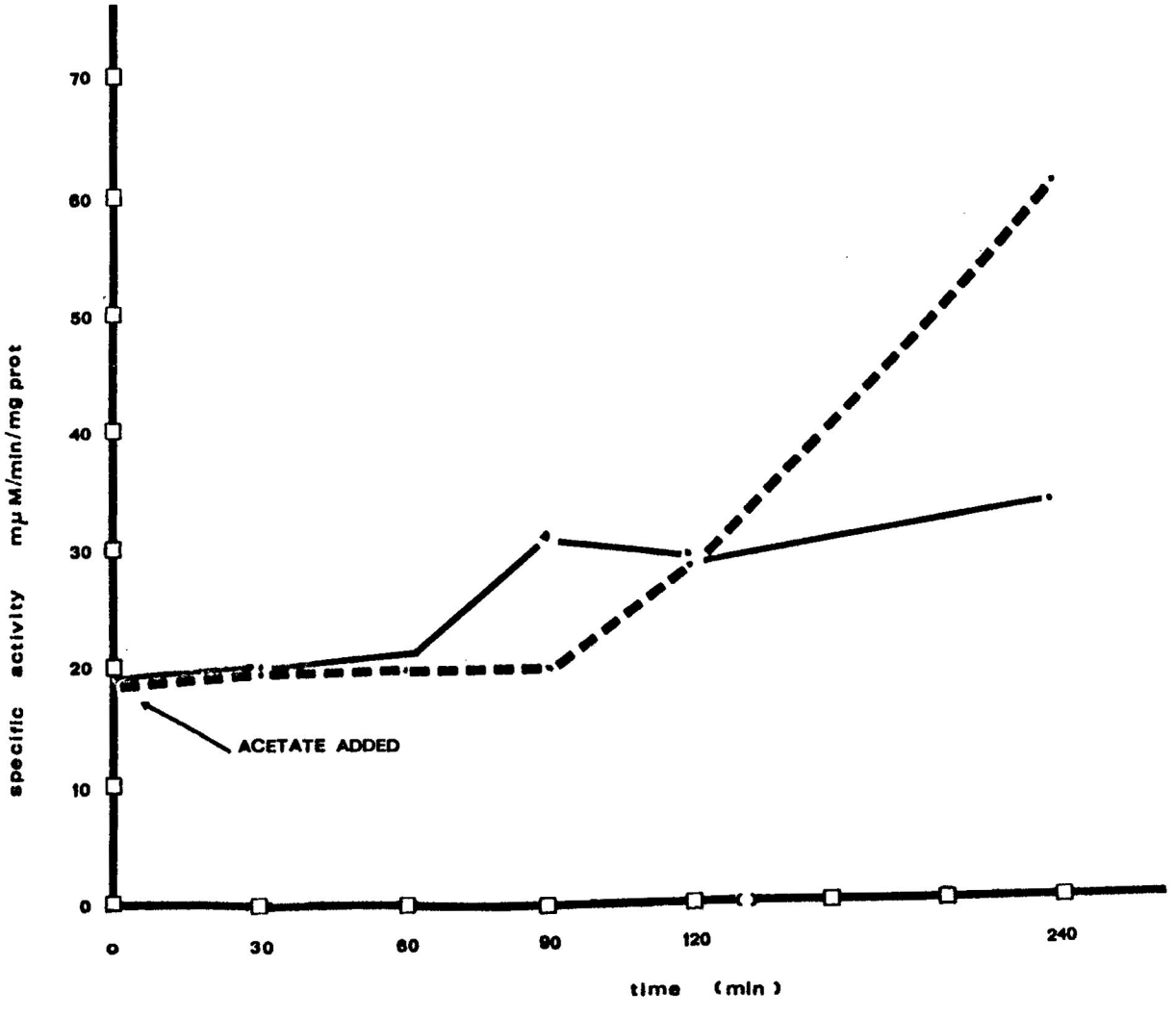

70
60
50
40
30
20
10
0
specific activity mμM/min/mg prot
ACETATE ADDED
o
30
60
90
120
240
time (min)

Fig. 5b. The effects of actinomycin D on the derepression of FDPase activity in cells of strain Y203. Cells were incubated for 2 hr in minimal media with (-- • --) and without (____ . _____) the addition of actinomycin D (80 μg/ml). Acetate (1.0%) was added to both cultures at the end of 2 hr pre-incubation.

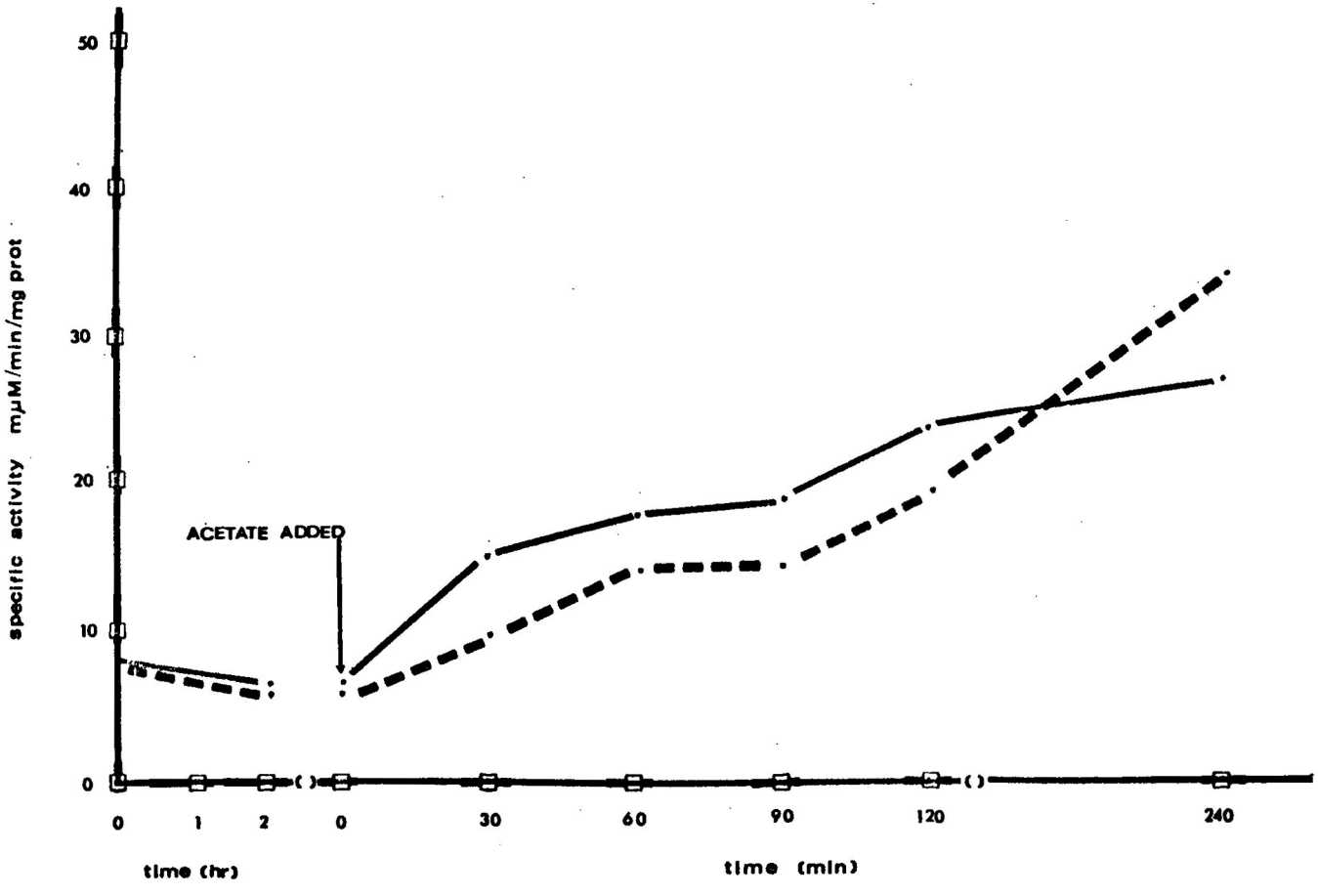
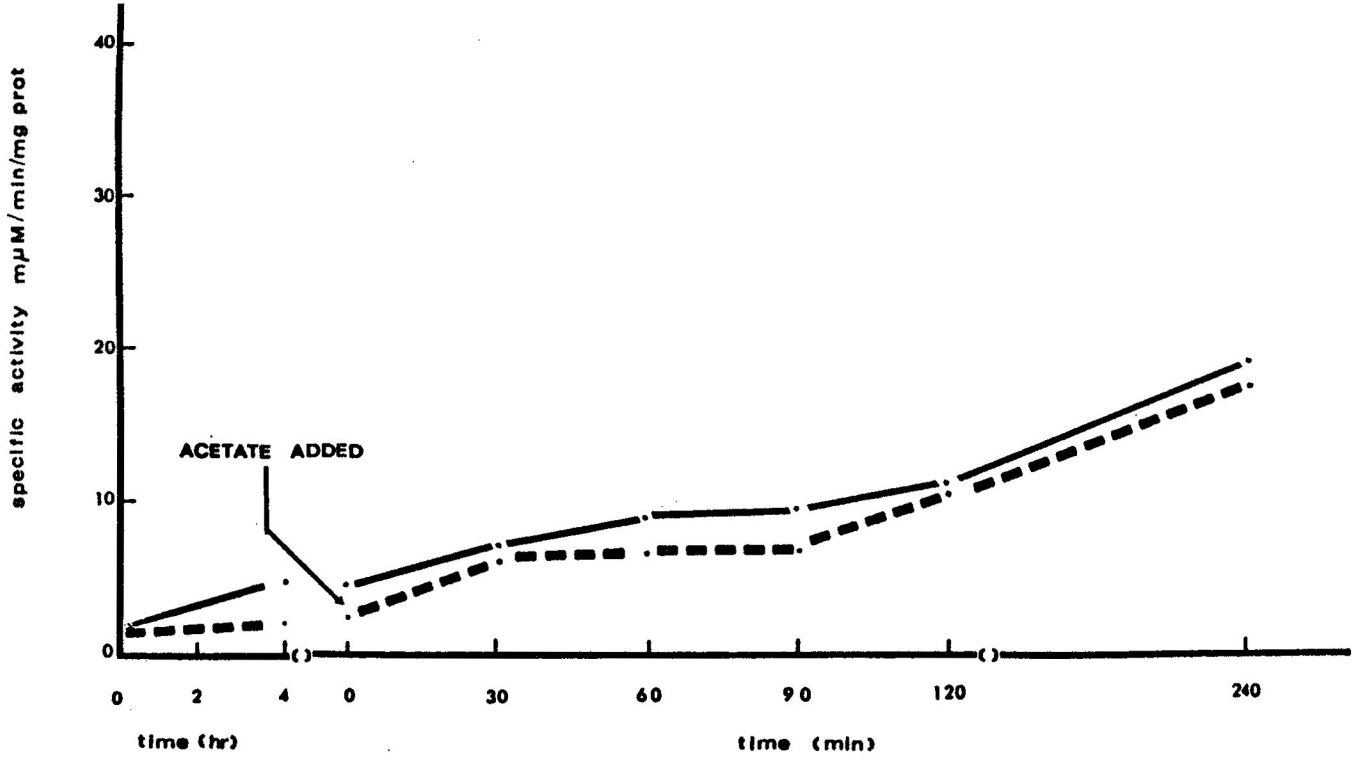

specific activity mμM/min/mg prot
50
40
30
20
10
0
ACETATE ADDED
0
1
2
0
30
60
90
120
240
time (hr)
time (min)

Fig. 5c. The effects of actinomycin D on the derepression of FDPase activity in acetate-induced cells of strain Y203. Cells were incubated for 4 hr in minimal media with (-- · --) and without (—— · ——) the addition of actinomycin D (80 μg/ml). Actate (1.0%) was added to both cultures at the end of 4 hr pre-incubation.


specific activity mμM/min/mg prot
40
30
20
10
0
ACETATE ADDED
0
2
4
0
30
60
90
120
240
time (hr)
time (min)

repressed conditions were incubated for 4 hr in minimal media with and without the addition of actinomycin D at 80 μg/ml (Fig. 5a). Acetate was added to both cultures and induction was allowed to proceed for 4 additional hr. While the control derepression curve appears somewhat typical, induction of FDPase in the presence of actinomycin D is inhibited for a short period of time (approx. 90 min). During the remaining intervals of the experiment, in which the activity of the enzyme rises slightly in the control curve, there appears to be a rapid burst of enzyme activity in the presence of actinomycin D. The final specific activity in the experimental curve was observed to be much greater than in the control. A priori, it appears that the inhibition of induction of FDPase by actinomycin D is released and that the cells recover the capacity to make the enzyme. Further interpretations of these findings will be discussed later.

Figure 5b represents a repeat of the procedures followed in Fig. 5a with one notable exception; cultures were incubated in minimal media with and without actinomycin D for only 2 hr before the addition of acetate rather than 4 hr. The initial inhibition of induced FDPase activity by actinomycin D seen in Fig. 5a is not seen in this experiment. This observation may reflect an inability of the antibiotic to permeate untreated (intact) cells effectively during a 2 hr exposure. However, the specific activity in the experimental curve is consistently below that of the control, indicating some interference with induction of FDPase. Further, a notable increase in specific activity of the enzyme occurs after approximately 90 min exposure to the carbon source, in the presence of actinomycin D. Again, the final activity

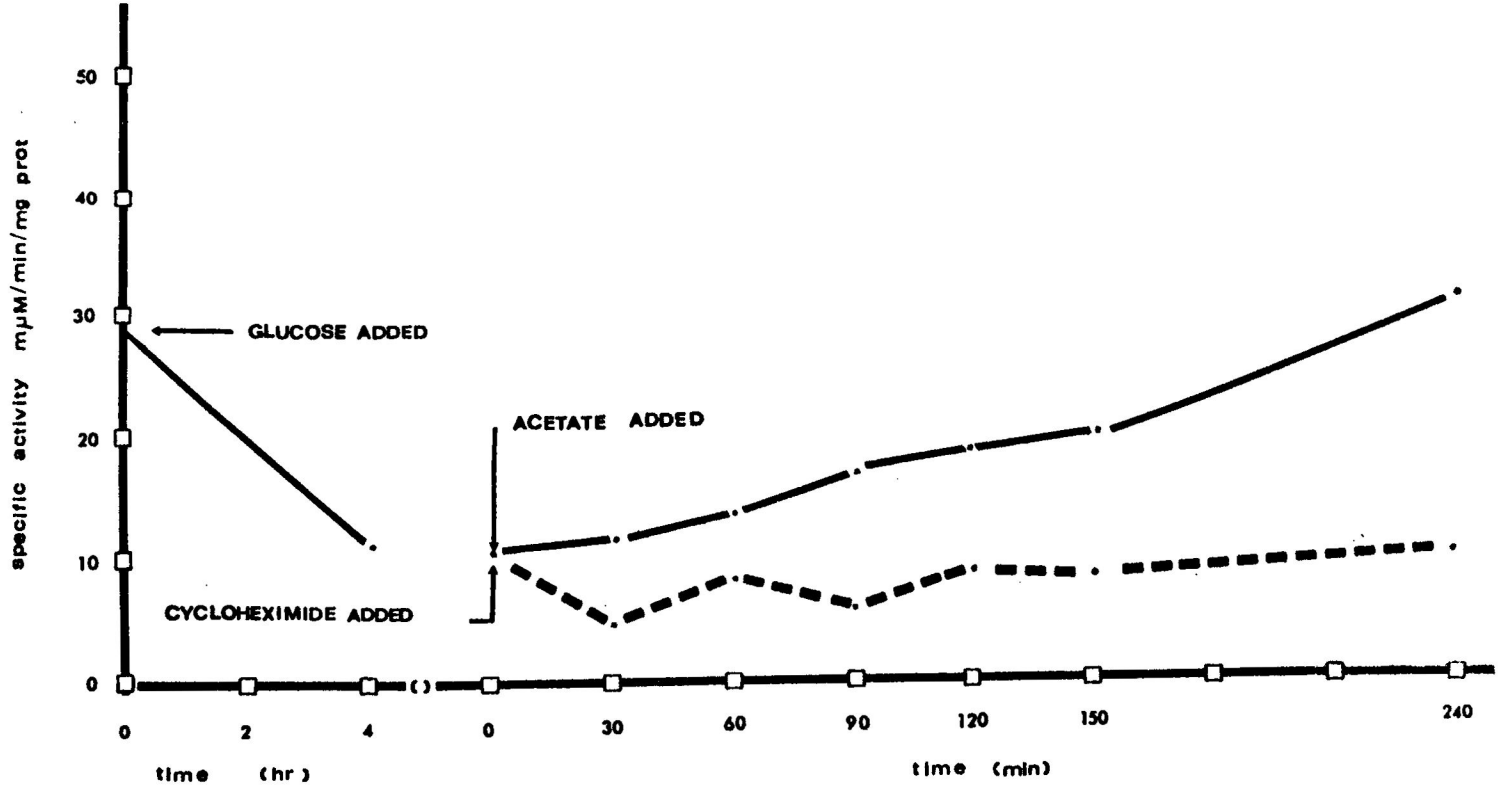
of the enzyme was greater in the presence, rather than in the absence, of the antibiotic.

The results shown in Fig. 5c were obtained after incubating cultures in minimal media again for 4 hr in the presence and absence of actinomycin D. The rise in activity seen in the control curve before the addition of acetate may possibly be attributed to the accumulation of endogenous metabolites or to products accumulated in the starvation medium. For my purposes, the rise was assumed to be insignificant. It is also observed that although actinomycin D does not prevent an initial rise in FDPase activity after the addition of the carbon source, the enzyme activity stabilizes thereafter for 1 hr. As was noted previously, there appears to be a release of the inhibition of FDPase activity in the presence of actinomycin D occurring at about the same time interval as noted before (Figs. 5a, 5b). Although the final activity in the experimental curve is not as high as in the control, the two final values are comparable. Tentatively, it may be concluded that actinomycin D inhibits induction of FDPase by acetate; that de novo synthesis of the protein is required to achieve maximal expression of the enzyme during derepression.

Effects of Cycloheximide on the Derepression of FDPase

Cycloheximide, a known inhibitor of protein synthesis in yeast and in other organisms, was found to inhibit or diminish the reappearance of FDPase in the presence of acetate (Fig. 5d). In this experiment, cultures of Y203 were induced initially in acetate, then grown under repressed conditions in glucose and finally induced again in acetate with and without the addition of the antibiotic, cycloheximide

Fig. 5d. The effects of cycloheximide on the derepression of FDPase activity in cells of strain Y203. Glucose (2.0%) was added to cultures pre-incubated for 4 hr in 1.0% acetate. The cultures were induced again in 1.0% acetate with (-- · --) and without (—— · ——) the addition of cycloheximide (1 μg/ml).


specific activity mμM/min/mg prot
50
40
30
20
10
0
GLUCOSE ADDED
ACETATE ADDED
CYCLOHEXIMIDE ADDED
0
2
4
0
30
60
90
120
150
240
time (hr)
time (min)

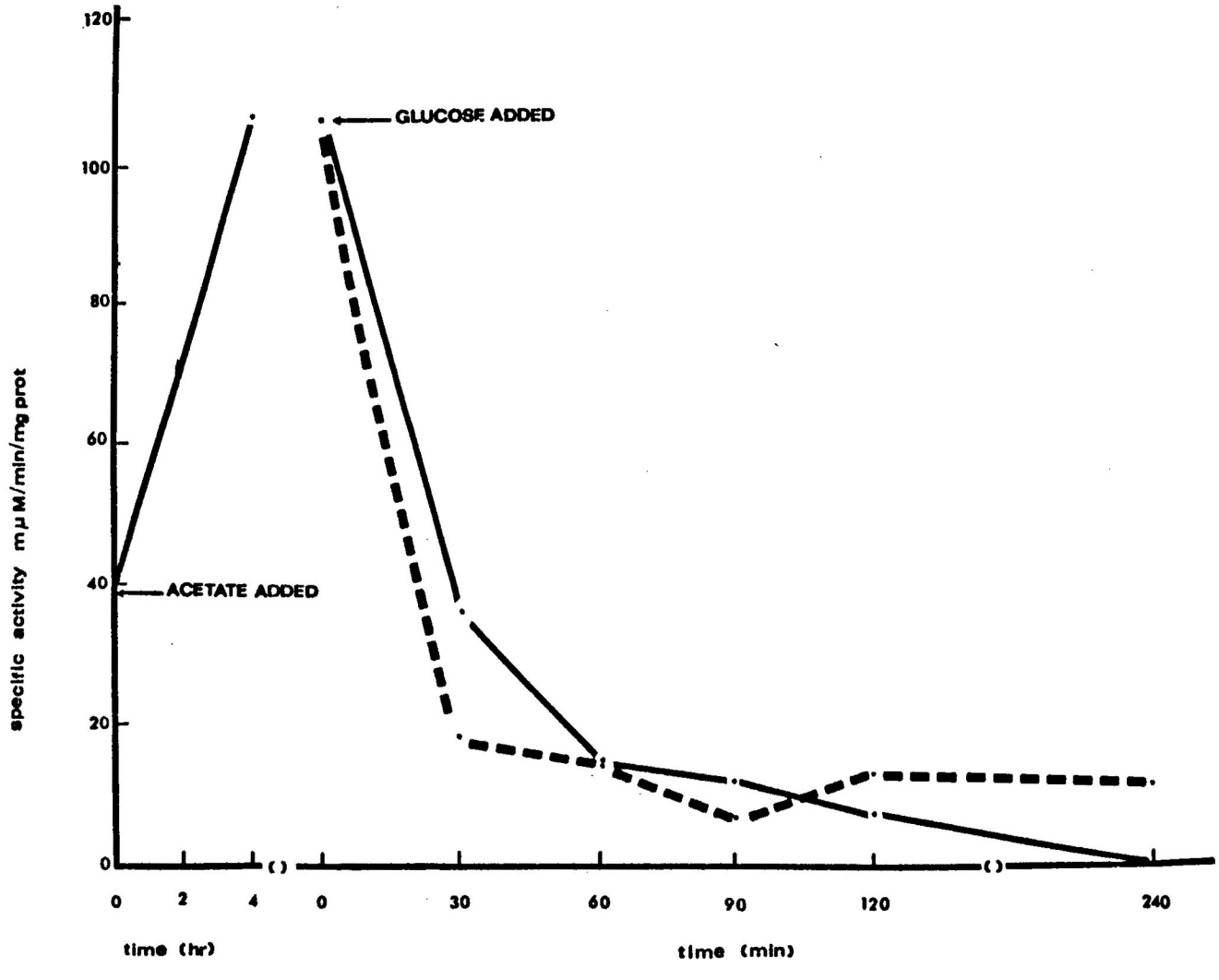
(1 μg/ml). Although the activity of the enzyme does oscillate somewhat in the experimental curve, the final specific activity after 4 hr induction in the presence of cycloheximide never surpasses the initial level found at the moment of its addition. However, the control derepression curve approaches an activity level comparable to the level seen before glucose addition. The curves in Fig. 5d thus indicate that the reappearance of FDPase after inactivation/repression by glucose requires de novo protein synthesis.

Effects of Actinomycin D on Inactivation/Repression by Glucose

The design of the next series of experiments was set so as to elucidate the possible level of protein synthesis, i.e., transcriptive or translative, at which inactivation/repression by glucose of FDPase activity occurs. From a glucose grown culture, cells of strain Y203 were induced in acetate for 4 hr then resuspended for 4 hr in media containing 2.0% glucose with and without actinomycin D (80 μg/ml). As seen in Fig. 6, glucose causes a rapid decline in the specific activity of FDPase which continues during 4 hr of incubation in the absence of actinomycin D. Such a rapid decline (first 30 min) suggests that glucose or one of its catabolites is directly or indirectly inactivating existing enzyme molecules rather than affecting the enzyme synthesis. The lower level of activity seen in 30 min after the addition of both the antibiotic and glucose indicates that actinomycin D does not prevent nor diminish the "glucose effect" but may be additive.

If during the latter stages of the experiment (Fig. 6) (30-240 min) the "glucose effect" depends partly on protein synthesis and since previous results indicate that there is a time-dependent release of the

Fig. 6. The effects of actinomycin D on the inactivation/repression of FDPase in strain Y203. Cultures were pre-incubated for 4 hr in 2.0% glucose followed by 4 hr of growth in induction media (acetate - 1.0%). The media was changed to 2.0% glucose and inactivation/repression was allowed to proceed for 4 hr in the presence (-- • --) and absence (——— • ———) of actinomycin D (80 µg/ml).


specific activity mμM/min/mg prot
120
100
80
60
40
20
0
GLUCOSE ADDED
ACETATE ADDED
0
2
4
0
30
60
90
120
240
time (hr)
time (min)

effects of actinomycin D on protein synthesis, this may account for the rise in activity seen in the presence of the antibiotic and glucose. That is, during early stages of inactivation/repression, actinomycin D diminishes both the synthesis of FDPase and the effects of glucose on FDPase which are mediated through the synthesis of proteins. The release of the actinomycin D effects would allow any existing mRNA specific for FDPase to be translated accounting for the slight rise and stabilization of enzyme activity. However, this interpretation implies that mRNA for FDPase is rather stable during inactivation/repression events.

Effects of Cycloheximide on Inactivation/Repression by Glucose

If cycloheximide (1 μg/ml) is added along with glucose to a previously induced culture of Y203, the inductive rise in specific activity ceases; however, the rapid decline in activity due to inactivation by glucose is no longer apparent. These results are seen in Fig. 7a. Although the activity of FDPase oscillates when glucoee alone is added, apparently, cycloheximide when added together with glucose diminishes glucose inactivation/repression during 2 hr of incubation.

The results in Fig. 7b indicate that even when cycloheximide is added 30 min prior to the addition of glucose, the inactivating effects on FDPase by glucose are not abolished. However, continued incubation with the antibiotic produces an activity of FDPase considerably higher than with glucose alone. Again, it is apparent that the initial effects of glucose are on enzyme inactivttion with later effects of glucose depending in part on protein synthesis.

Figure 7c shows the results obtained when cycloheximide is added

Fig. 7a. The effects of cycloheximide on the inactivation/repression of FDPase in strain Y203. Cultures were pre-incubated for 4 hr in 1.0% acetate (0-time activity). The media was changed to 2.0% glucose and inactivation/repression was allowed to proceed for 2 hr in the presence (-- • --) and absence (—— • ——) of cycloheximide (1 μg/ml).

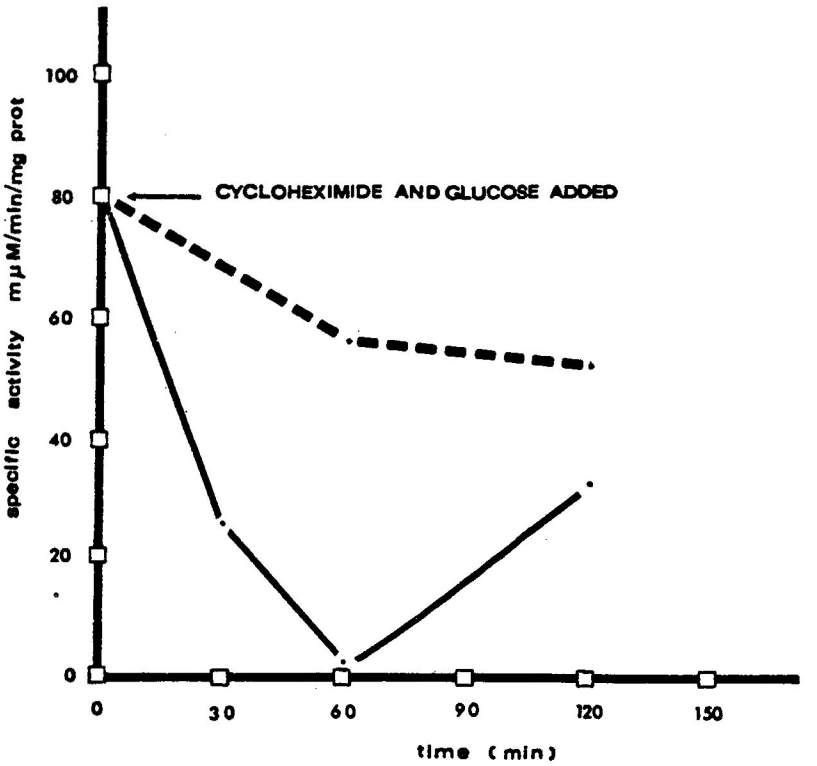

specific activity mμM/min/mg prot
100
80
60
40
20
0
CYCLOHEXIMIDE AND GLUCOSE ADDED
0
30
60
90
120
150
time (min)

Fig. 7b. A graph of the effects of cycloheximide on the inactivation/repression of FDPase. Cultures were pre-incubated for 4 hr in 1.0% acetate (0-time activity) followed by the addition of cycloheximide (1 µg/ml) 30 min prior to glucose (2.0%) addition (-- • --). Also presented is a control curve of inactivation/repression by glucose (2.0%) alone (—— . ——).

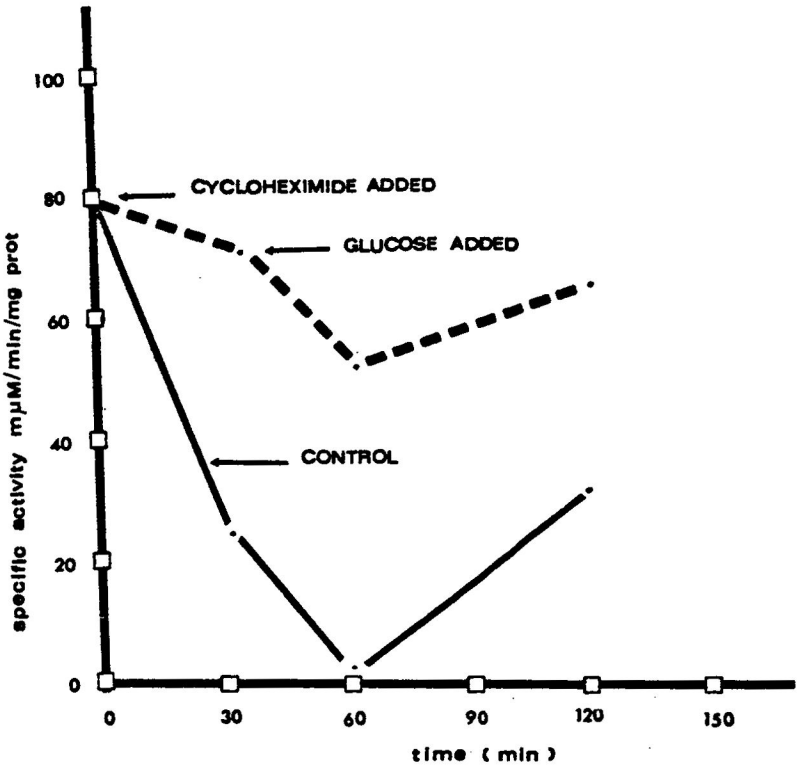
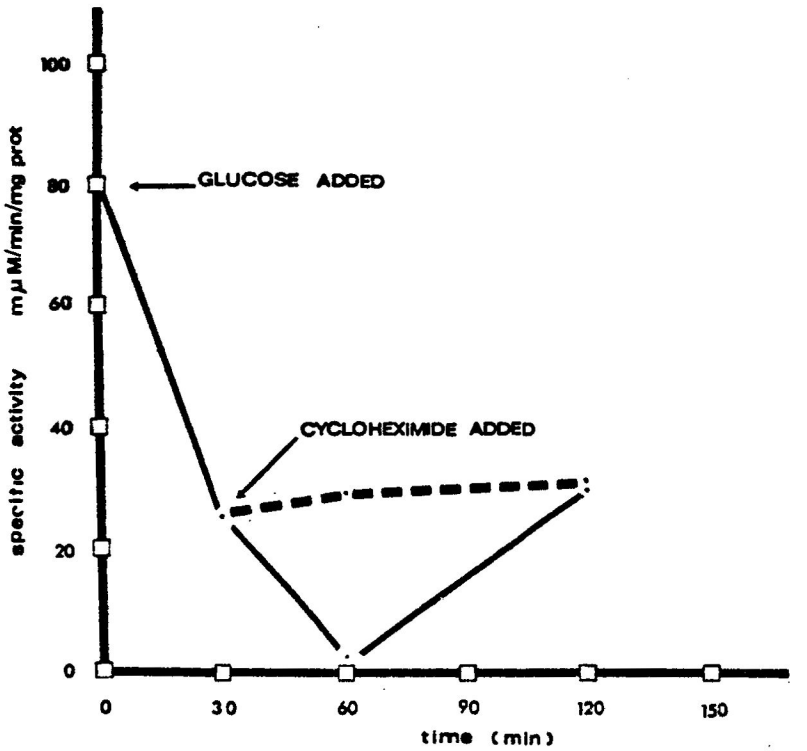

100
80
60
40
20
0
specific activity mμM/min/mg prot
CYCLOHEXIMIDE ADDED
GLUCOSE ADDED
CONTROL
0
30
60
90
120
150
time (min)

Fig. 7c. A graph of the effects of cycloheximide on the inactivation/repression of FDPase. Cultures were pre-incubated for 4 hr in 1.0% acetate (0-time activity) followed by the addition of 2.0% glucose (—— · ——) 30 min prior to cycloheximide (1 μg/ml) addition (-- · --).


100
80
60
40
20
0
specific activity mμM/min/mg prot
GLUCOSE ADDED
CYCLOHEXIMIDE ADDED
0
30
60
90
120
150
time (min)

to cultures 30 min after the addition of a repressive concentration of glucose (2.0%). These results further suggest that the later phases of the "glucose effect" most probably are mediated through an effect on protein synthesis. After cycloheximide addition, no further decline nor substantial increase in enzyme activity is noted.

The question now arises relative to what effects on the activity of existing FDPase are mediated by cycloheximide. The results from Fig. 7d show that even in a culture that oscillates in its protein synthetic ability, cycloheximide prevents further increases in protein synthesis without severely limiting the expression of the existing enzyme.

Effects of Various Glucose Concentrations on Inactivation/Repression

Cultures of Y203 were grown under repressed conditions (2.0% glucose) and resuspended in an acetate (1.0%) medium for 4 hr followed by suspension again in media containing various glucose concentrations. The results are observed in Figs. 8a and 8b. From both figures, it appears that the initial effects of glucose during inactivation of FDPase are dependent on the glucose concentration of the medium. Concentrations of 0.01, 0.05, 0.10, 0.5, 1.0 and 2.0% glucose all produced a decline in activity of the enzyme during the initial 30 min interval tested. It should be noted that the higher the initial glucose concentration, the greater appears the initial decline; however, after 30 min, no further conclusions can be drawn. The writer chose 2.0% glucose as the standard concentration to demonstrate inactivation/repression simply based on its consistent behavior when compared to the other concentration tested.

Fig. 7d. The effects of cycloheximide on the induced level of FDPase. Cultures were pre-incubated for 4 hr in 1.0% acetate (0-time activity). Cycloheximide (1 μg/ml) was added to the cultures in the absence of a carbon source (-- • --). Also presented is a control curve of inactivation/repression by glucose (2.0%) alone (—— • ——).

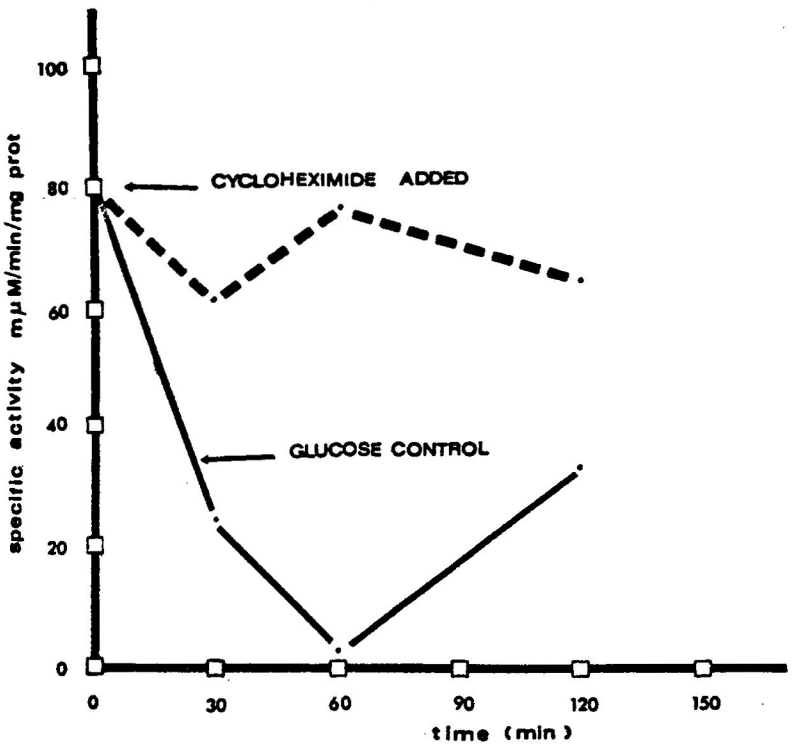

100
80
60
40
20
0
specific activity mμM/min/mg prot
CYCLOHEXIMIDE ADDED
GLUCOSE CONTROL
0
30
60
90
120
150
time (min)

Fig. 8a. The effects of various glucose concentrations on the induced level of FDPase. Cultures were induced in 1.0% acetate for 4 hr followed by resuspension in media containing the following glucose concentrations: 0.01% (—— · ——), 0.05% (--·· --), and 0.10% (——···——).

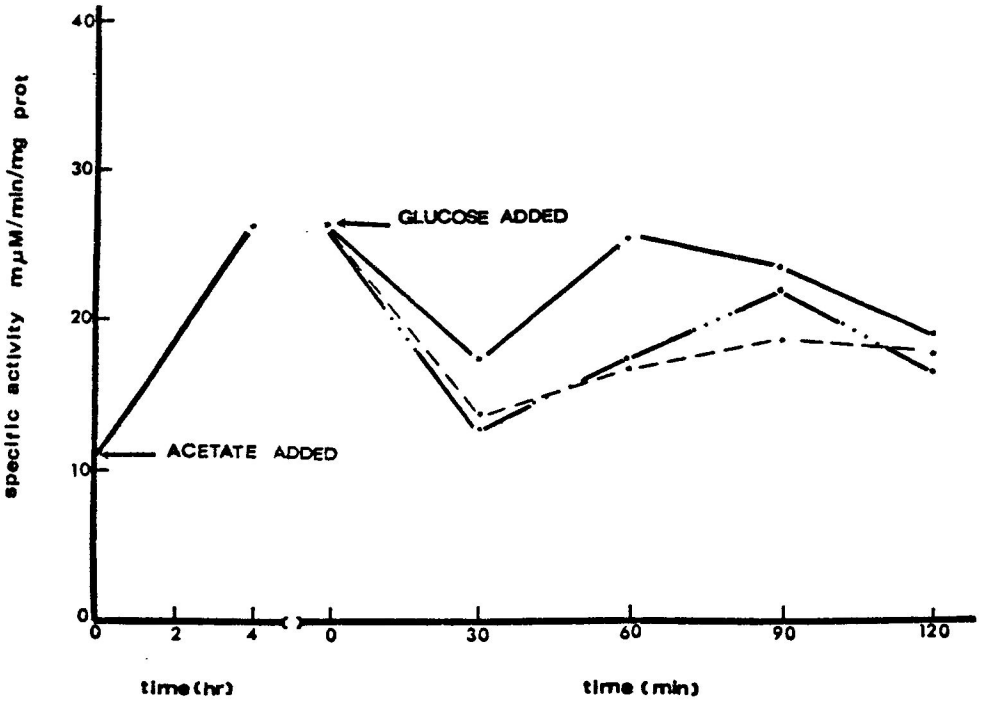
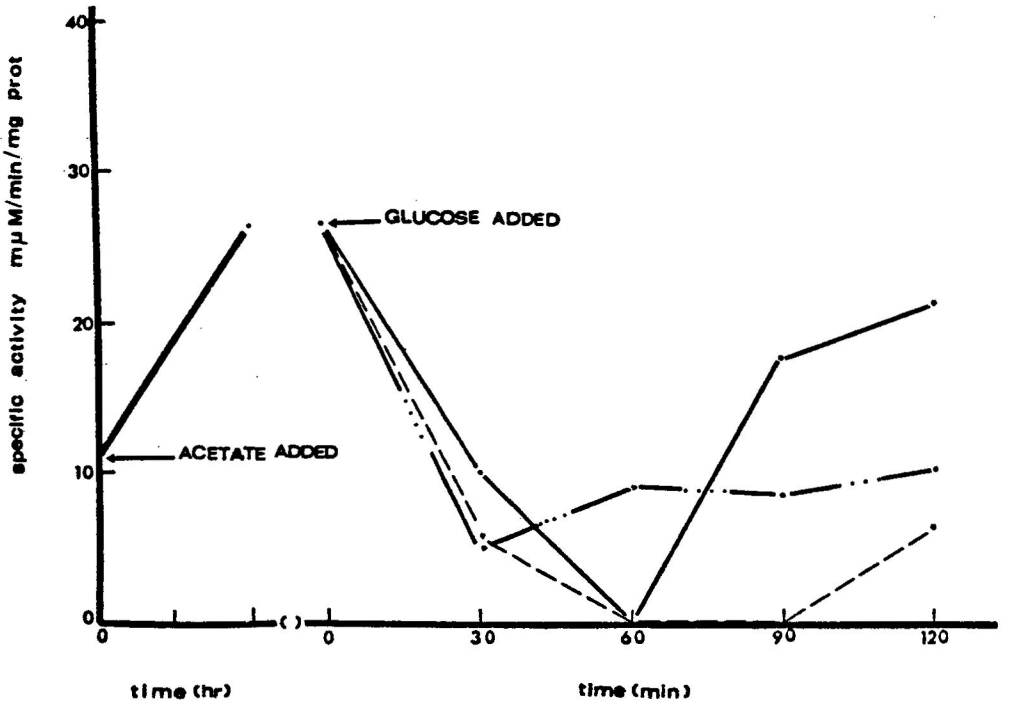

specific activity mμM/min/mg prot
40
30
20
10
0
GLUCOSE ADDED
ACETATE ADDED
0
2
4
0
30
60
90
120
time (hr)
time (min)

Fig. 8b. The effects of various glucose concentrations on the induced level of FDPase. Cultures were induced in 1.0% acetate for 4 hr followed by resuspension in media containing the following glucose concentrations: 0.50% (—— . ——), 1.0% (-- · --), and 2.0% (·——···——·).


specific activity mμM/min/mg prot
40
30
20
10
0
ACETATE ADDED
GLUCOSE ADDED
0
0
30
60
90
120
time (hr)
time (min)

Effects of Metabolic Inhibitors on Inactivation/ Repression by Glucose

Since cycloheximide, a known inhibitor of protein synthesis, appeared to diminish or prevent repression of FDPase by glucose, the experiments in Figs. 9a, 9b, and 9c were performed in attempts to inhibit the "glucose effect" with other metabolic inhibitors. Fig. 9a illustrates that known inhibitors of energy-yielding metabolism, sodium azide, sodium fluoride, and iodoacetamide, all at 1 mM concentration did not prevent the initial decline in activity of FDPase produced by glucose. However, a rise in activity of the enzyme is seen during the latter portions of the experiment only with the addition of iodoacetamide. It is postulated that the initial "glucose effect" is mediated via direct enzyme inactivation with later effects requiring in part and affecting protein synthesis. The later effects of glucose on repression appear here to require an energy source because iodoacetamide prevents a further decline in enzyme activity and the enzyme activity stabilizes in the presence of sodium azide and sodium fluoride.

Fig. 9b indicates that when iodoacetamide is added together with glucose and sodium fluoride, an initial decline in activity is still evident, however, an established rise in activity is again produced. Evidently, iodoacetamide addition overcomes the repressive effects on FDPase produced by glucose. An analogue of glucose, 2-deoxy-D-glucose, at 0.01% concentration also was able to produce an initial decline in the activity of FDPase but was not as effective as glucose over the course of the experiment. Fructose (1.0%) also elicited a decline in

Fig. 9a. The effects of various metabolic inhibitors on glucose-mediated inactivation/repression of FDPase activity. Cultures of Y203 were induced for 4 hr in acetate (1.0%). Induced cells were resuspended in 2.0% glucose alone (—— . ——) and with the following metabolic inhibitors: sodium azide (1 mM) (-- · --), sodium fluoride (1 mM) (·——···——·), and iodoacetamide (1 mM) (·————————·).

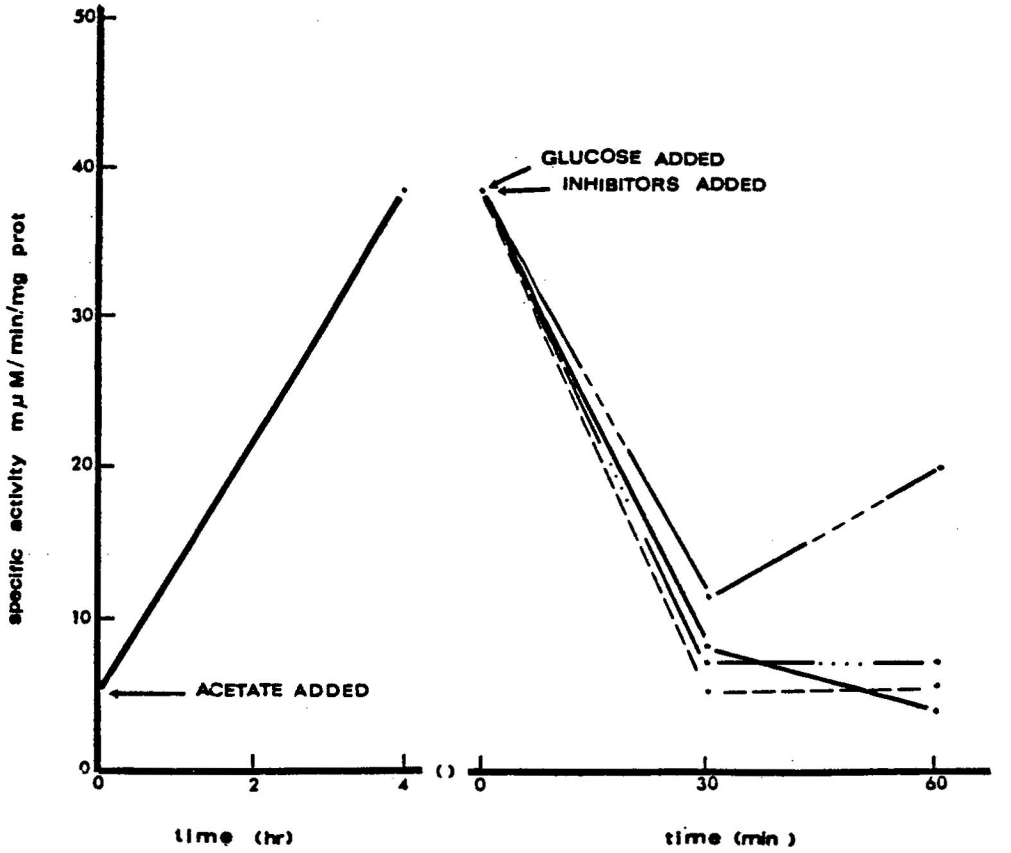

specific activity mμM/min/mg prot
50
40
30
20
10
0
ACETATE ADDED
GLUCOSE ADDED
INHIBITORS ADDED
0
2
4
0
30
60
time (hr)
time (min)

Fig. 9b. The effects of various metabolic inhibitors on the induced level of FDPase. Cultures of Y203 were induced for 4 hr in acetate (1.0%). Induced cells were resuspended in 2.0% glucose alone (—— · ——) and together with two inhibitors, iodoacetamide (1 mM) and sodium fluoride (1 mM) (-- · --). Also, the effects of 2-deoxy-D-glucose (0.01%) (·——···——·) and 1.0% fructose (·————·) without the addition of glucose are illustrated.

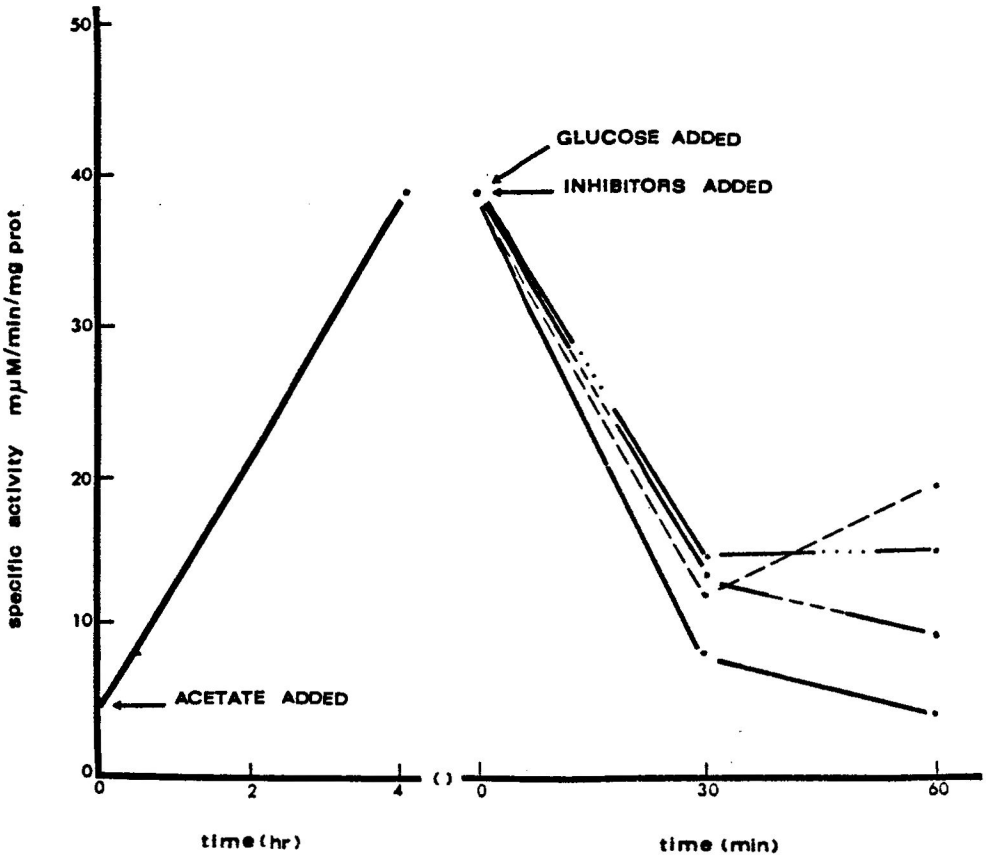
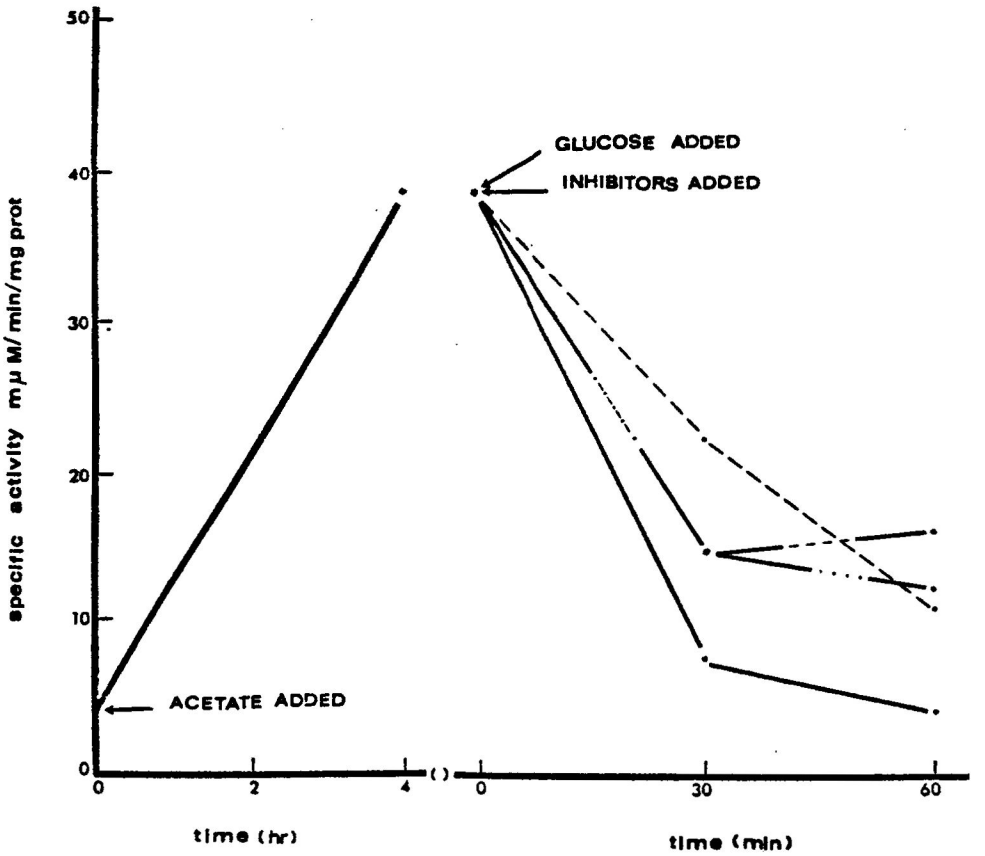

specific activity mμM/min/mg prot
50
40
30
20
10
0
GLUCOSE ADDED
INHIBITORS ADDED
ACETATE ADDED
0
2
4
( )
0
30
60
time (hr)
time (min)

Fig. 9c. The effects of various metabolic inhibitors on the induced level of FDPase. Cultures of Y203 were induced for 4 hr in acetate (1.0%). Induced cells were resuspended in 2.0% glucose alone (—— . ——) and with 10 mM p-fluorophenylalanine (.——...——.) or 5 mM 5-methyl tryptophane (.——---——.). Also are presented the effects of 1.0% mannose (-- • --) without the addition of glucose.

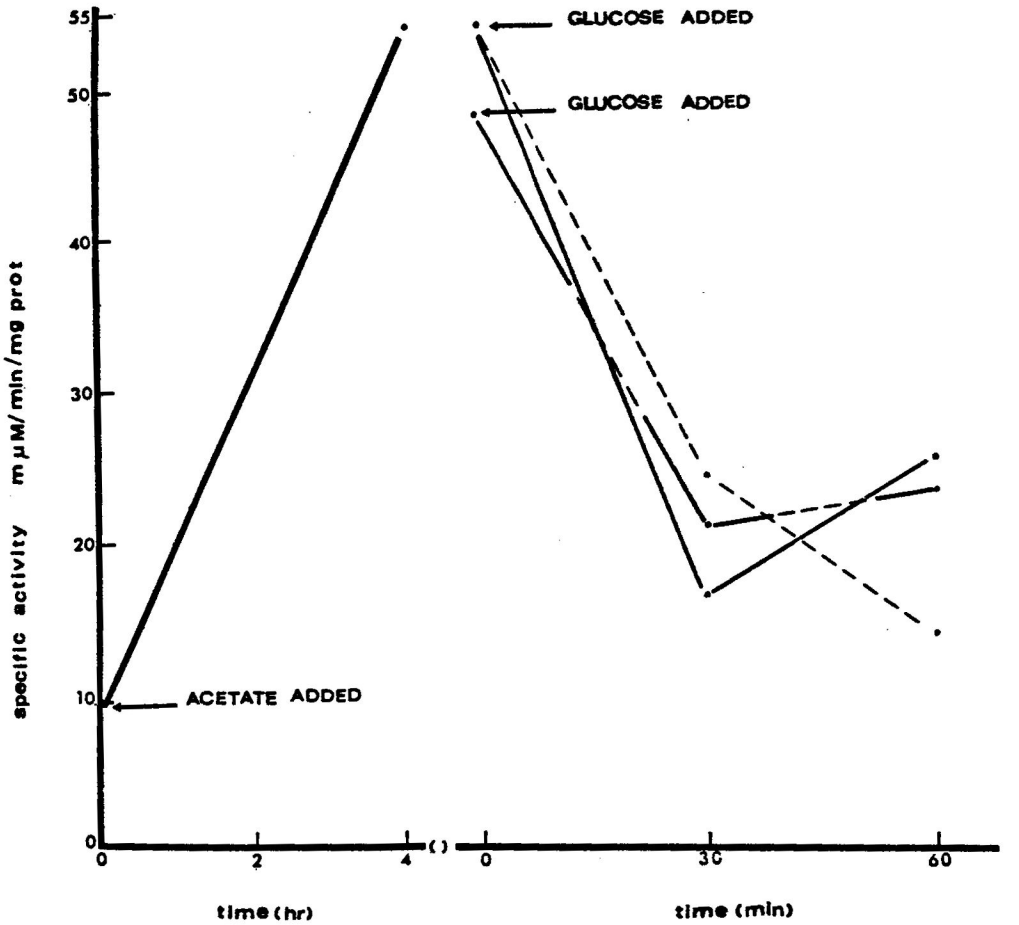

specific activity mμ M/min/mg prot
50
40
30
20
10
0
GLUCOSE ADDED
INHIBITORS ADDED
ACETATE ADDED
0
2
4
time (hr)
0
30
60
time (min)

the enzyme activity. Evidence of this sort indicates that glucose itself is not the metabolite which represses the activity of FDPase. The amino acid analogues, p-fluorophenylalanine (10 mM) and 5-methyl tryptophane (5 mM) did not prevent the inactivating effects of glucose. However, the data in Fig. 9c indicate that some interference with the "glucose effect" is occurring during the latter intervals tested in the presence of these two amino acid analogues. At the 30 min interval checked, the inactivation in the presence of the two analogues is not as great as with glucose alone. Further, at 1 hr of incubation, the activity of the enzyme even rises in the presence of 5-methyl tryptophane and glucose. Results are consistent with the interpretation that prolonged effects of glucose on the activity of FDPase depend in part and affect de novo protein synthesis.

Effects of Nitrogen Starvation on the Inactivation/ Repression of FDPase

Figure 10 shows the results obtained in an experiment designed to study the effects of nitrogen starvation on the inactivation of FDPase. Strain Y203 was cultured under two different nitrogen starvation conditions: (1) Cells previously induced in acetate (1.0%) were resuspended in minimal media without nitrogen but containing 2.0% glucose; (2) another induced culture was incubated in minimal media (with trace amounts of biotin) for 4 hr before the addition of glucose, however, still in the absence of a good nitrogen source. A normal glucose inactivation curve is also presented. This series of experiments did not provide a wealth of useful information. Although the enzyme activity did fluctuate in the glucose control curve, a more

Fig. 10. A graph of the effects of nitrogen starvation on glucose-mediated inactivation/repression of FDPase activity. Cultures of Y203 were induced in 1.0% acetate for 4 hr and resuspended in minimal media with glucose (2.0%) but without a nitrogen source (-- • --). Cultures also were incubated without nitrogen or glucose (.———---———.) with glucose (2.0%) being added at the end of 4 hr incubation. Also presented is a control inactivation curve with 2.0% glucose alone (——— . ———).


GLUCOSE ADDED
GLUCOSE ADDED
ACETATE ADDED
specific activity mμM/min/mg prot
55
50
40
30
20
10
0
0
2
4
0
30
60
time (hr)
time (min)

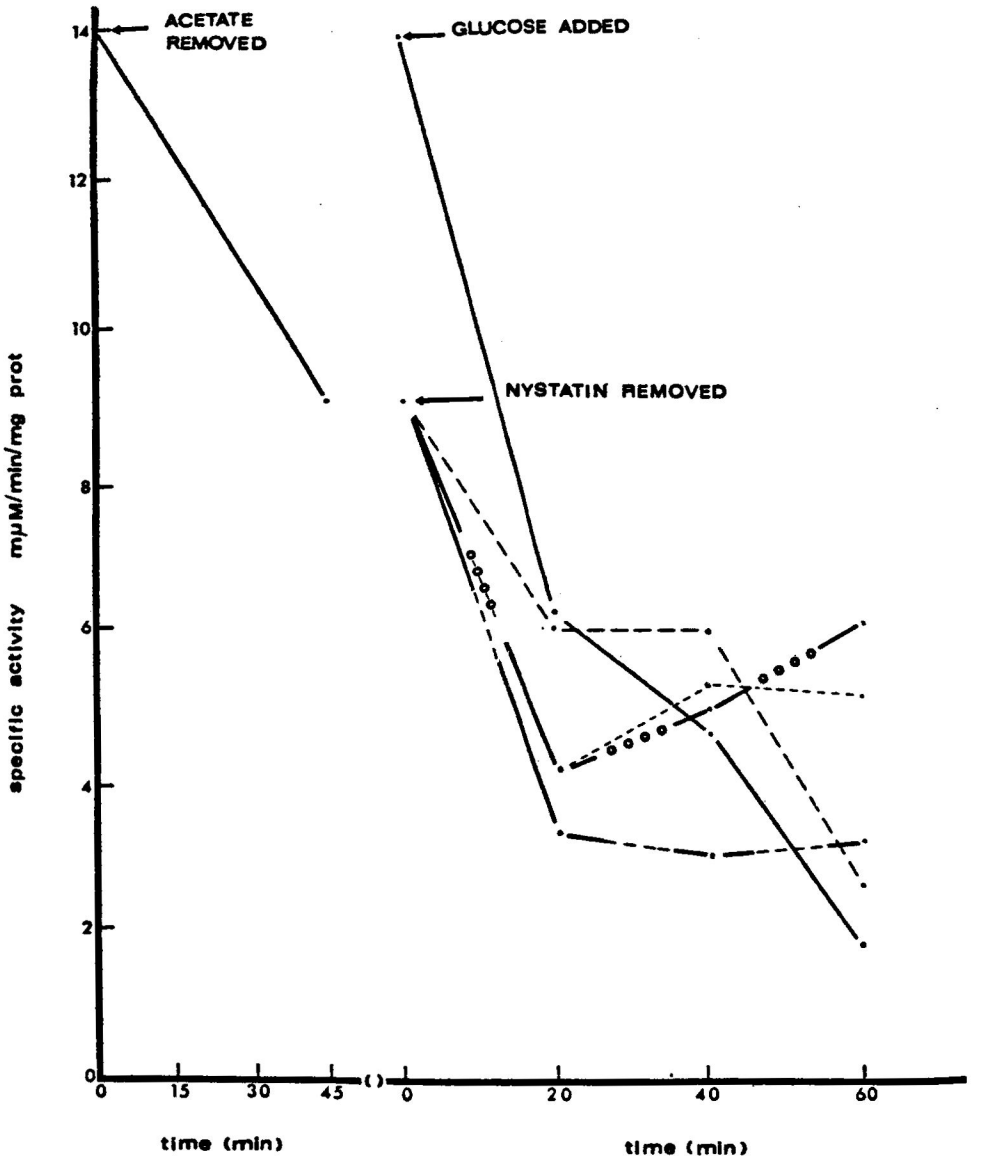
drastic type of inactivation was observed in the absence of a nitrogen source but with the immediate addition of glucose. This type of result would be expected since de novo synthesis of FDPase would require nitrogen, and if the inactivation phenomenon were independent of protein synthesis. The rise in enzyme activity observed 30 min after glucose addition in the 4 hr nitrogen-starvation curve may implicate residual translation of FDPase. This would mean that mRNA for FDPase synthesis was formed after induction by acetate, stored during the initial stages of nitrogen starvation and remained stable during glucose inactivation.

Effects of Phosphorylated Sugars on Inactivation/ Repression of FDPase

In experiments testing the effects of phosphorylated sugars on the inactivation of FDPase, cultures of Y203 were induced in 1.0% acetate for 4 hr and resuspended for 45 min in 0.9% NaCl containing 65 µg/ml of nystatin at a cell concentration of 150 mg dry wt/ml of media. The nystatin treatment was performed to increase the permeability of the cells to various phosphorylated sugars. Cells were harvested from the permeabilizing medium and resuspended with the various sugars presented in Fig. 11a all at 1.0% concentration. The reduction in activity of the enzyme after nystatin treatment repeatedly could not be abolished. Also presented in the figure is a curve reflecting the "glucose effect" with and without prior nystatin treatment.

The results seen in Fig. 11a indicate that nystatin treatment diminishes the overall effects of glucose in producing a decline in FDPase activity. The effects noted may be due to interference by

Fig. 11a. The effects of various phosphorylated sugars on the induced level of FDPase. Cells of strain Y203 were pre-incubated for 4 hr in 1.0% acetate and treated with nystatin (65 μg/ml) for 45 min. Harvested cells were incubated with various sugars all at 1.0% concentration. (—— . ——), glucose added without prior nystatin treatment. The following sugars were added after nystatin pre-treatment: glucose (--•--), glucose 6-phosphate (.——————.), fructose 6-phosphate (.——oooo——.), and 3-phosphoglyceric acid ( ).


ACETATE REMOVED
GLUCOSE ADDED
NYSTATIN REMOVED
specific activity mμM/min/mg prot
14
12
10
8
6
4
2
0
0
15
30
45
0
20
40
60
time (min)
time (min)

nystatin of the glucose permease system associated with the cell membrane. The decline in activity seen 20 min after the addition of glucose 6-phosphate and 3-phosphoglyceric acid (3-PGA) are probably not due to inactivation by the sugars but to residual effects of nystatin. As will be seen later, cells suspended in minimal media alone after nystatin treatment also show an initial decline at 20 min. Results indicate that only glucose 6-phosphate produces a continued repressive effect on the activity of FDPase while fructose 6-phosphate and 3-PGA appear to be somewhat inductive in their responses.

In an effort to reproduce the effects of glucose 6-phosphate on the repression of FDPase activity, the same procedures as in Fig. 11a were followed again, except the initial induction period was increased to 8 hr. The results in Figs. 11b and 11c were obtained and, in addition, the behavior of fructose 6-phosphate was monitored again. Further, the response of alpha-glycerophosphate is illustrated. Figures 11b and 11c illustrate that the initial decline in activity seen at 20 min of incubation after nystatin treatment could not be prevented by any of the sugars tested. Further, the addition of glucose without prior nystatin treatment (Fig. 11b) produced a more repressive response on the activity of FDPase for the duration of the experiment than did the other sugars. Again, it appears that nystatin treatment interferes with glucose utilization (compare curves of glucose added with and without prior nystatin treatment). The results in Fig. 11b further illustrate that glucose 6-phosphate is capable of repressing the activity of FDPase which remains stable after the initial "nystatin effect".

Fig. 11b. The effects of glucose 6-phosphate on the induced level of FDPase. Cultures were pre-incubated for 8 hr in acetate (1.0%) and treated with nystatin (65 µg/ml) for 45 min. Harvested cells were incubated with glucose (1.0%) (-- · --) and glucose 6-phosphate (1.0%) (.——————.). Also presented is a control curve of glucose (1.0%) addition without prior nystatin treatment (—— . ——).

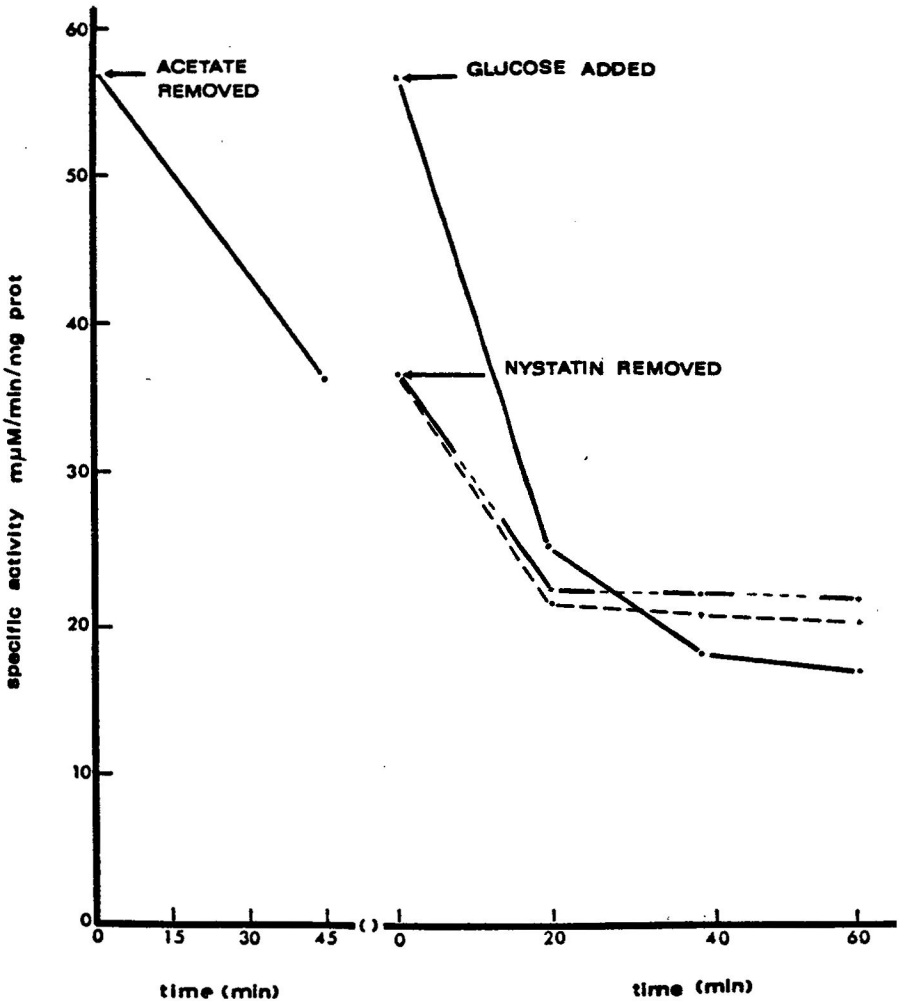
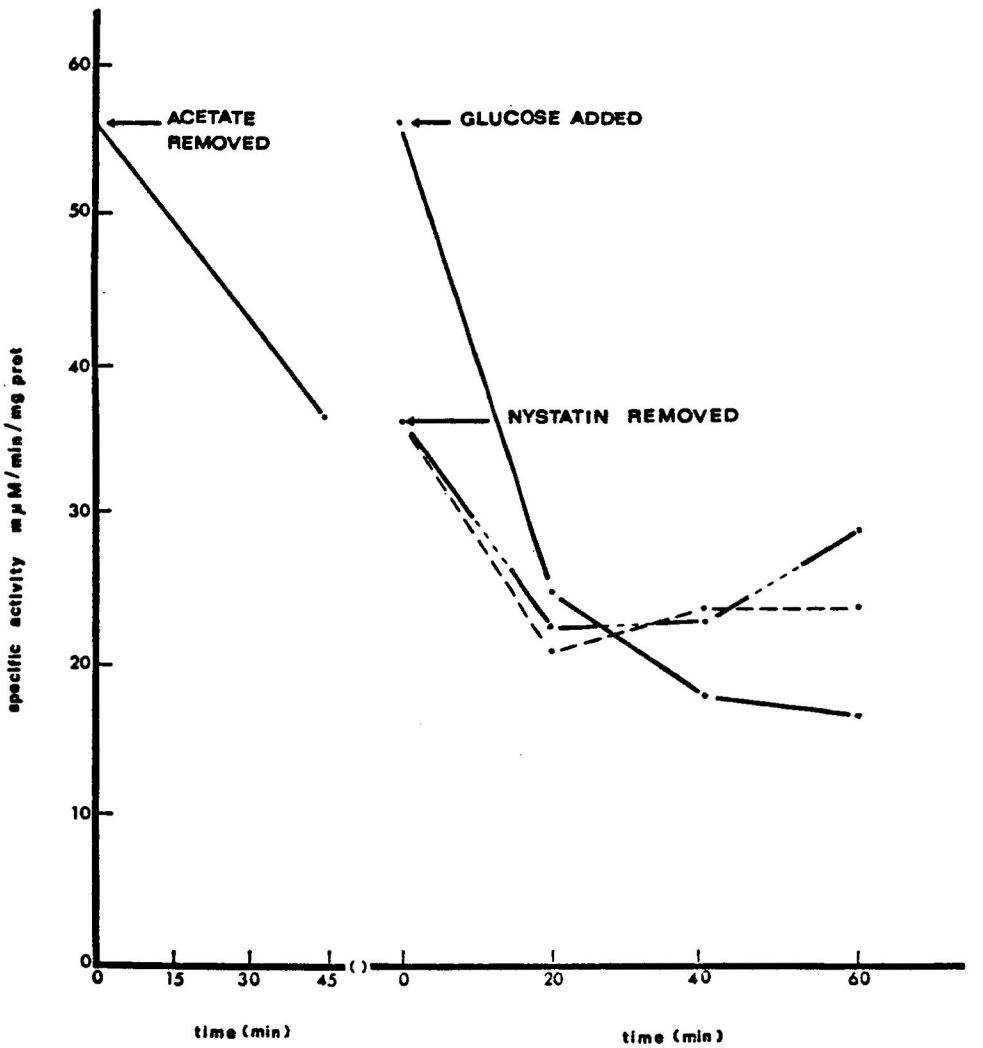

ACETATE
REMOVED
GLUCOSE ADDED
NYSTATIN REMOVED
specific activity mμM/min/mg prot
60
50
40
30
20
10
0
0
15
30
45
0
20
40
60
time (min)
time (min)

Fig. 11c. The effects of phosphorylated sugars on the induced level of FDPase. Cultures were pre-incubated for 8 hr in acetate (1.0%) and treated with nystatin (65 µg/ml) for 45 min. Harvested cells were incubated with alpha-glycerophosphate (1.0%) (•——————•) and fructose 6-phosphate (1.0%) (-- • --). A glucose (1.0%) control inactivation curve is presented (—— • ——).

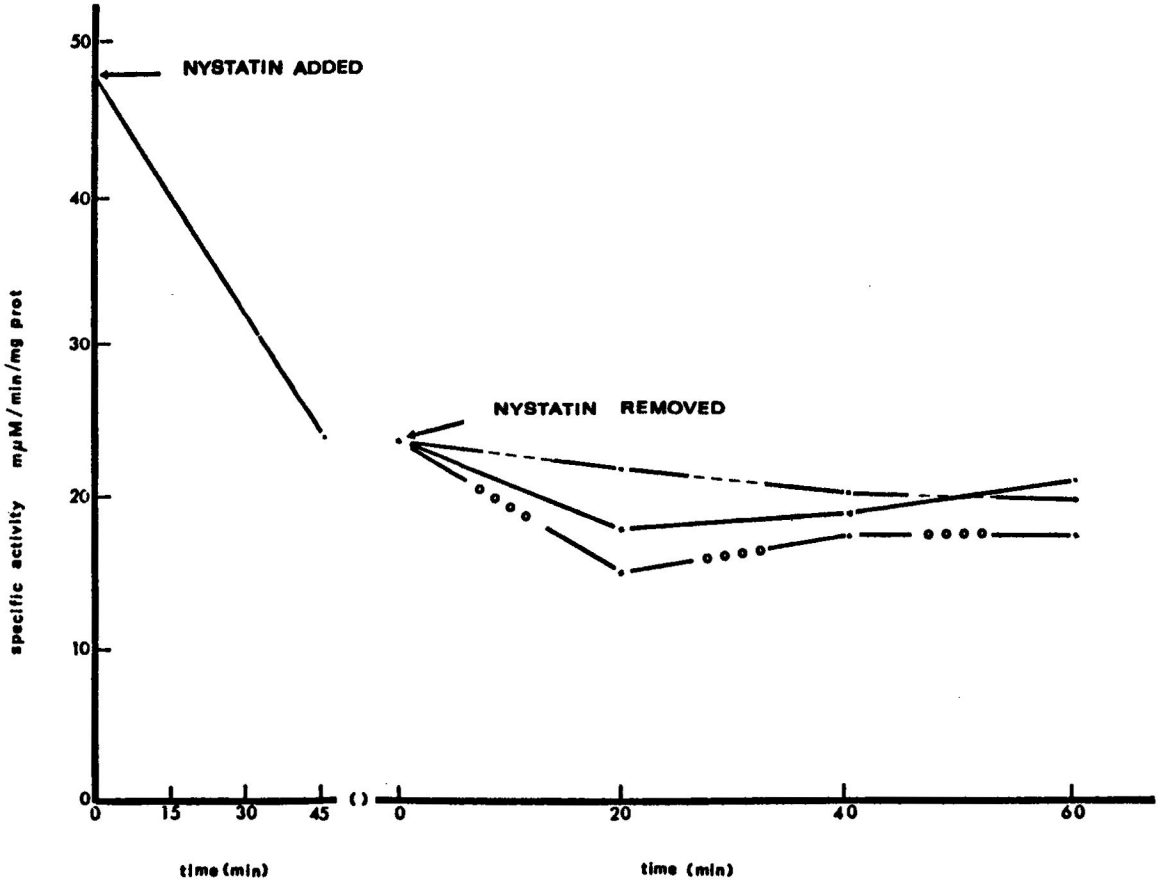

ACETATE REMOVED
GLUCOSE ADDED
NYSTATIN REMOVED
specific activity mμM/min/mg prot
time (min)
time (min)
0
10
20
30
40
50
60
0
15
30
45
0
20
40
60

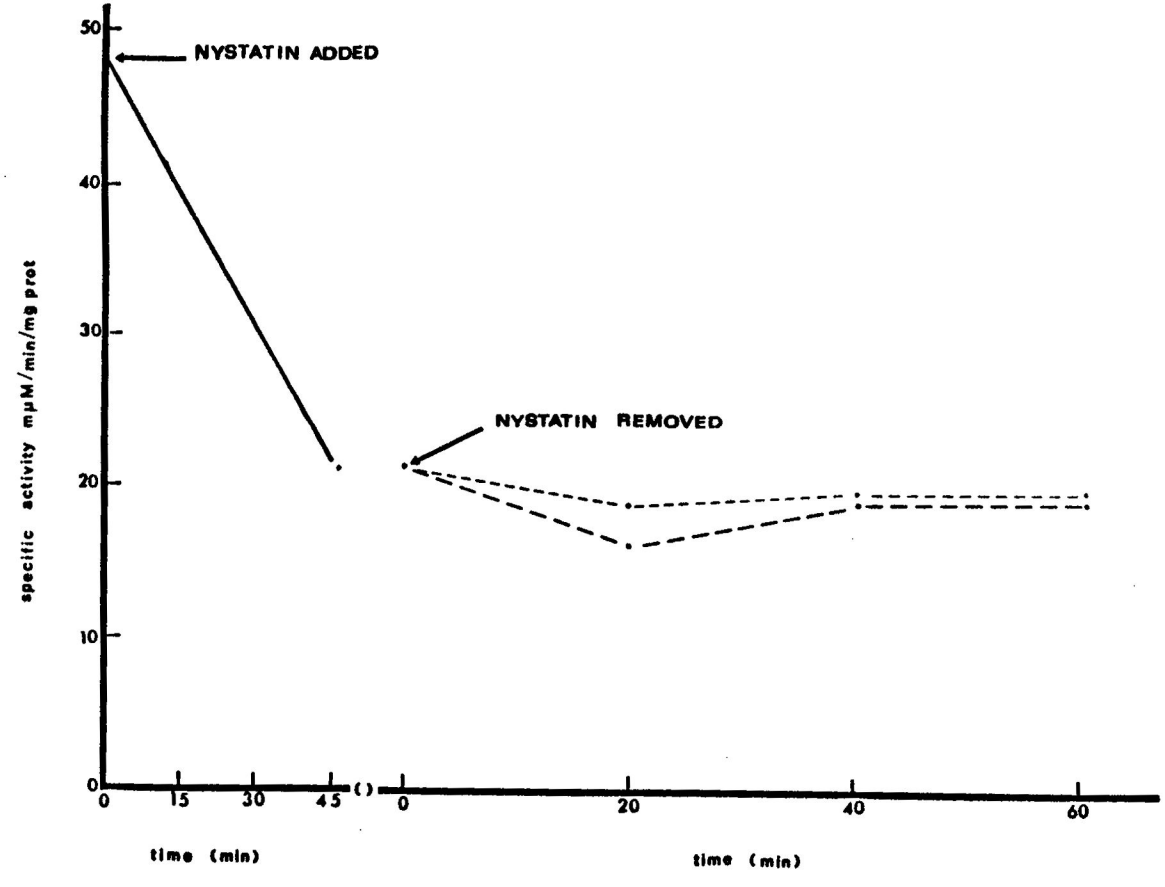
Observations from Fig. 11c indicate that again fructose 6-phosphate is not repressive nor is alpha-glycerophosphate.

Since the aliquots of cells obtained after nystatin treatment in Figs. 11b and 11c were collected by filtration, the filtrates were collected, chilled and assayed for protein content and FDPase. In none of the filtrates could a measurable activity of FDPase be detected. However, at all intervals (20, 40 and 60 min), a moderate amount of protein was found. This indicates that nystatin treatment produces some leakage of cellular contents during permeabilization. This may account for the less intense effect of glucose addition after prior nystatin treatment when compared to its addition without pre-treatment of the cells. However, it is doubtful that pre-treatment with nystatin accounts for the non-repressive effects of the other sugars tested. Leakage of cellular contents may, on the other hand, account for the initial drop in activity (20 min response) after nystatin treatment seen in all samples checked.

Efforts were now made to check the inductive influence of acetate (1.0%) after nystatin treatment. The nystatin treatment was performed as before after 8 hr of induction in acetate. The representative results are shown in Figs. 12a and 12b which illustrate that again the initial drop in activity occurs after treatment of the cells with the antibiotic. When cells were suspended in minimal media without a carbon source, the enzyme activity rises and stabilizes after the "nystatin effect". Phosphoenolpyruvate is not able to produce a measurable decline in enzyme activity (between 20 and 60 min); however,

Figs. 12a and 12b. The effects of phosphorylated sugars on the induced level of FDPase. Cultures of Y203 were pre-incubated for 8 hr in 1.0% acetate and resuspended in nystatin (65 µg/ml) media for 45 min. Harvested cells were incubated with the following compounds all at 1.0% concentration: acetate (—— . ——), phosphoenol-pyruvate (--•--), glucose (•————————•), and glucose 6-phosphate (.——oooo——.). (— —.— —) is the behavior of FDPase in minimal media alone.


NYSTATIN ADDED
NYSTATIN REMOVED
specific activity mμM/min/mg prot
50
40
30
20
10
0
0
15
30
45
0
20
40
60
time (min)
time (min)


NYSTATIN ADDED
NYSTATIN REMOVED
specific activity mμM/min/mg prot
50
40
30
20
10
0
0
15
30
45
0
20
40
60
time (min)
time (min)

glucose addition led to a continued repressive effect on the specific activity of FDPase. The effects of glucose 6-phosphate, although not as stabilizing as previously shown, did produce the lowest activity level. Acetate, on the other hand, produced a continued rise in enzyme activity up to 1 hr of the incubation experiments. However, acetate addition did not alleviate the initial drop in activity presumably produced by the nystatin treatment.

Since nystatin treatment can produce undesirable effects on yeast cells, the experiments to follow were performed without prior treatment of cells. To further pursue the effects of glucose 6-phosphate on FDPase activity, cultures of Y203 were induced for 4 hr in acetate and resuspended for 1 hr in glucose (1.0%), glucose 6-phosphate (1.0%), and minimal media without a carbon or nitrogen source. The observations from these procedures are noted in Fig. 13 with an identical experiment being repeated in Fig. 14. In addition, the changes in cell density at the intervals tested were monitored. In both Figs. 13 and 14, glucose produced an immediate decline in activity noted here 20 min after the addition of the carbon source. The activity of the enzyme oscillates for the remaining 40 min; however, marked increases in cell growth were noted throughout the exposure to glucose. Cultures incubated without a carbon source (Figs. 13 and 14) did not illustrate a further increase in activity after removal of acetate but a gradual decline during 1 hr of incubation. Further, cells in minimal media without a carbon source did not grow as evident by the absence of a rise in the cell densities.

Fig. 13. Optical density changes and FDPase activity during growth under repressive conditions. Cultures were incubated for 4 hr in acetate (1.0%) and transferred to: glucose (1.0%) (—— . ——), glucose 6-phosphate (1.0%) (— —.— —), and minimal media without a carbon source (.————————.). Cell densities were recorded (O.D. 400μ; 10^2 dilution) at 20 min intervals. The initial cell density for all cultures was 0,710.

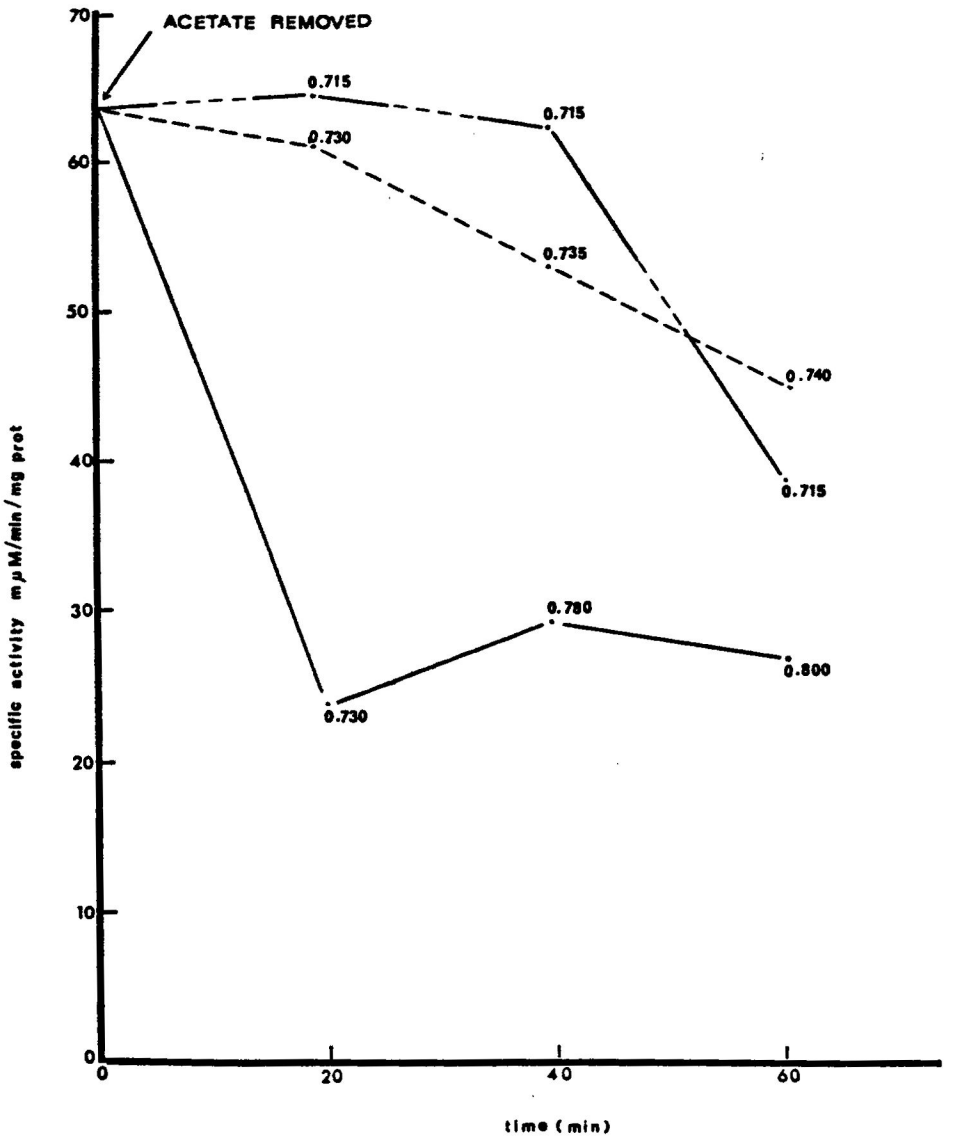
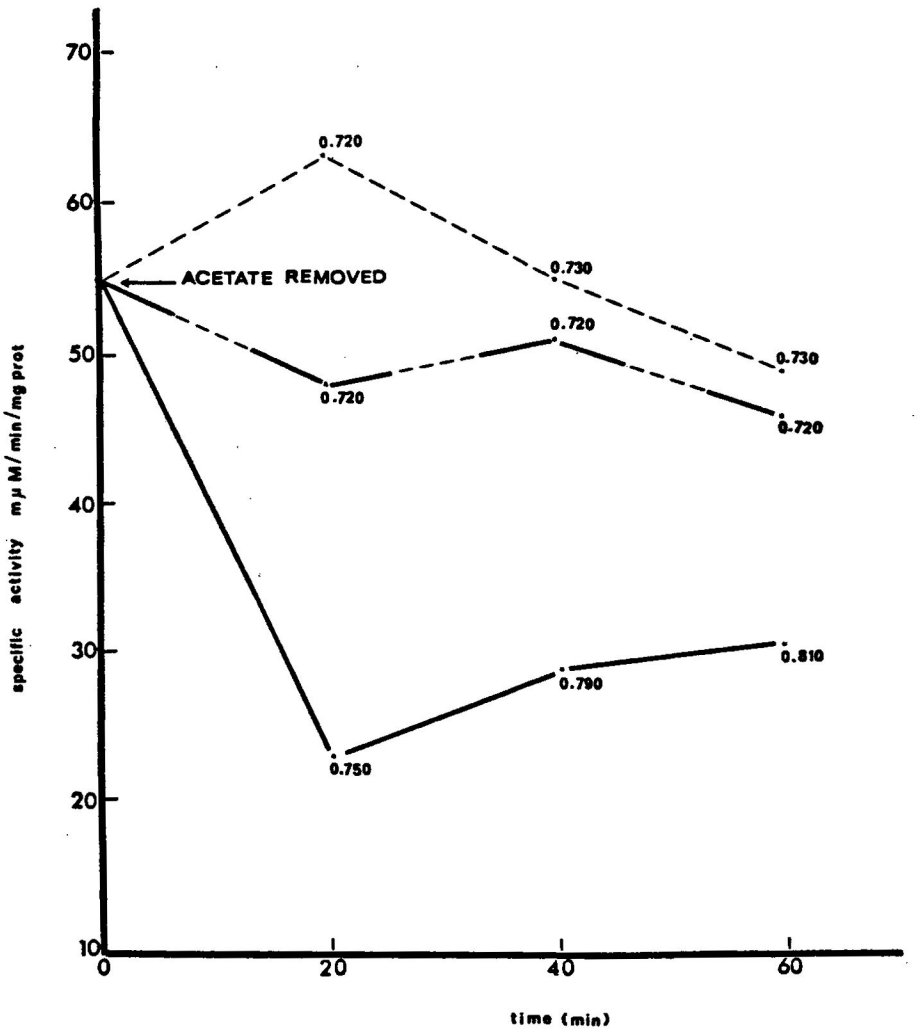

ACETATE REMOVED
0.715
0.715
0.730
0.735
0.740
0.715
0.730
0.780
0.800
specific activity mμM/min/mg prot
time (min)
0
10
20
30
40
50
60
70
0
20
40
60

Fig. 14. Optical density changes and FDPase activity during growth under repressive conditions. Cultures were pre-incubated for 4 hr in 1.0% acetate and transferred to: glucose (1.0%) (—— . ——), glucose 6-phosphate (1.0%) (— —·— —), and minimal media alone (•——————•). Cell densities were recorded (O.D. 400μ; 10^2 dilution) at 20 min intervals. The initial cell density for all cultures was 0.720.


70
60
50
40
30
20
10
0
20
40
60
specific activity mμM/min/mg prot
time (min)
ACETATE REMOVED
0.720
0.730
0.730
0.720
0.720
0.720
0.750
0.790
0.810

Data in Figs. 13 and 14 indicate that Y203 does not grow appreciably in glucose 6-phosphate. However, the specific activity of FDPase does decline over the 1 hr incubation period which is most notable 20-60 min after the addition of the sugar phosphate. While the final specific activities at 1 hr of incubation in minimal alone are comparable to those obtained in the presence of glucose 6-phosphate, the data do not exclude the latter compound as a contributory catabolite in glucose inactivation/repression of FDPase activity.

CHAPTER V

DISCUSSION AND CONCLUSIONS

The present experimental results showed that in the yeast, Saccharomyces cerevisiae, FDPase is inducible (derepressible) during growth on acetate or pyruvate as sources of carbon. An examination of the kinetics of the increase in activity (derepression) for strain Y203 showed a time-dependent rise in the specific activity of FDPase when the yeast were suspended in media containing either acetate or pyruvate. The time course of induction being similar for both carbon sources.

Studies have shown that after induction, upon addition of glucose to the culture media, a rapid decline in specific activity of FDPase occurs. The decline in specific activity (inactivation/repression) was noted to be much more rapid than the corresponding acetate or pyruvate-mediated increases. It should be stated that the terms derepression (induction) and inactivation/repression are used in a functional sense only; that is, the phenomena described by these terms may not be quite analogous to situations described in procaryotic organisms. Biochemical events involving the rapid disappearance or inactivation of enzymes upon addition of glucose to the media have been described in yeast by Robertson and Halvorson (1957), Witt et al. (1966), Ferguson et al. (1967), and Gorts (1969). However, neither the chemical nature of the compound(s) which is (are) responsible for the "glucose effect" (Witt et al., 1966) nor its mode of action is known.

In S. cerevisiae, four gluconeogenic enzymes, alcohol dehydrogenase, malate dehydrogenase, isocitrate lyase, and malate synthetase, were found to undergo repression by glucose (Witt et al., 1966). These authors assumed that repression of the synthesis of the four enzymes is not caused by metabolites but by glucose itself, glucose 6-phosphate, or by changes in the concentration of adenosine 5'-triphosphate. However, glucose alone is not likely to be directly involved, according to the data of Sols (1967). His report indicates that the intracellular concentration of glucose in yeast is kept very low by the effects of a regulated transport system and also an excess of low Km hexokinase. Data are presented in this thesis which indicates that glucose itself is not likely to be directly involved in inactivation-repression of FDPase. For instance, results here illustrate that fructose or mannose can prevent derepression of FDPase activity; however, the disappearance of activity is not as rapid as with glucose.

The antibiotic, actinomycin D, can inhibit the induced synthesis of a variety of enzymes in many organisms. Results of the present investigation suggest that actinomycin D inhibits or diminishes induction of FDPase by acetate. Further, it is concluded that de novo synthesis of the protein is required to achieve maximal expression of the enzyme during derepression. The work of Sussman (1965) is pertinent to the conclusions drawn in this study. Sussman found that the appearance of UDP-galactose polysaccharide transferase in the slime mold, Dictyostelium discoideum, required de novo protein synthesis which could be prevented by actinomycin D. Actinomycin D was added four hr in advance of the normal appearance during morphogenesis of

this enzyme. Weber et al. (1965) studied the regulation of four gluconeogenic enzymes involved in the homeostatic control of blood sugar in rats. The enzymes, glucose 6-phosphatase, FDPase, PEP carboxykinase, and pyruvate carboxylase, were inducible by the glucocorticoid hormone. The increase in activities of all four gluconeogenic enzymes was prevented by actinomycin and the authors assumed that de novo enzyme synthesis occurred during induction.

Many observations (Exton et al., 1973) suggest that changes in adenosine 3', 5'-monophosphate (cyclic AMP) metabolism may be involved in the effects of insulin and diabetes on hepatic gluconeogenesis. Insulin administered to rats made diabetic by antiserum or alloxan lowers liver cyclic AMP (cAMP) and gluconeogenesis. Topping and Mayes (1972) have concluded that insulin decreases the formation of cAMP either by inhibition of adenylate cyclase or by enhancing the breakdown of cAMP by activating 3', 5'-cyclic nucleotide phosphodiesterase. Although a direct role of cAMP in transcriptional regulatory processes often goes lacking, Fontana and Lovenberg (1973) provide evidence that cAMP has an indirect role. In their study, phosphorylation of calf thymus chromatin was accomplished by cAMP-activated protein kinase from bovine pineal glands. This phosphorylation results in an apparent decrease binding between histones and the DNA in the chromatin as evidenced by an increase in actinomycin D binding sites after phosphorylation. Further, an increase in the template capacity of calf-thymus chromatin after phosphorylation was noted. Thus, cAMP may play an indirect rôle in the differential transcription of genes. In addition, Snell and Walker (1973) have observed that the activity of another

gluconeogenic enzyme, PEP carboxylase is markedly stimulated by glucagon or an analogue of cAMP, dibutyryl cyclic AMP. Induction of PEP carboxylase in rats *in utero*, immediately newborn, and in foetal liver transplants could be blocked by the simultaneous addition of actinomycin D.

Cyclic AMP has further been found to activate gluconeogenesis in the perfused rat liver (Jefferson et al., 1968; Exton and Park, 1968) and increases the activity of certain gluconeogenic enzymes (Exton et al., 1972). One of the glucocorticoids, cortisol, *in vivo* or *in vitro* significantly increases glucose output and gluconeogenesis from high or physiological levels of lactate in livers from adrenalectomized rats (Exton et al., 1973). Cortisol treatment, however, did not modulate the levels of cAMP nor the level of acetyl-CoA, an allosteric activator of an important gluconeogenic enzyme, pyruvate carboxylase (Utter and Keech, 1963).

It is interesting to note that the *in vitro* effects of cortisol on increased gluconeogenesis could be abolished by the addition of actinomycin D and cycloheximide. Thus, Exton et al. (1973) have suggested that the direct effects of glucocorticoids on hepatic glucose output and gluconeogenesis probably involve increased mRNA and protein synthesis. Further, there was no evidence of glucocorticoid action on FDPase or phosphofructokinase. Hence, the steroid does not apparently facilitate the net conversion of fructose 1,6-diphosphate to fructose 6-phosphate, the reaction of primary concern in the present investigation. However, the present investigator has observed that at 0.2 mM, a concentration at which 5'-AMP was found to inhibit FDPase activity

in crude extracts of S. cerevisiae at least 70%, equimolar concentrations of cAMP were without effect.

The present work provides evidence that cells of S. cerevisiae recover the capacity to synthesize FDPase after inhibition of induction by actinomycin D. Sawicki and Godman (1972) have reported that Vero cells, after pulse exposure to concentrations of actinomycin D sufficient to abolish transcription (1 μg/ml), recover the capacity to synthesize RNA much more rapidly than most cell types. Voll and Leive (1970) have isolated a mutant of E. coli which recovers RNA synthetic capacity rapidly. In both cell types, Vero cells and E. coli, bound actinomycin is excreted. Although the present investigator does not imply that S. cerevisiae can excrete bound actinomycin D, although necessary, this alone would be insufficient to explain the rapid resurgence of FDPase activity after inhibition by this antibiotic. Whatever mechanism responsible for actinomycin D excretion must be able to reverse the tight binding of DNA and the antibiotic (Pederson and Robbins, 1972) and must be functional at the time of exposure to actinomycin D. The reason for this assertion is that enzyme induction is virtually impossible in the absence of RNA synthesis.

Cycloheximide, an antifungal antibiotic, inhibits protein synthesis in a wide range of organisms including fungi, algae, protozoa, higher plants and animals (Siegel and Sisler, 1964a). Siegel and Sisler (1964b) have suggested that the cycloheximide site of action apparently involves the transfer of amino acyl-soluble RNA to the ribosomes without affecting amino acid activation or "charging" of tRNA. However, more recently, McKeehan and Hardesty (1969) described the effects of

cycloheximideiin eucaryotic cells, reticulocytes, as blocking the movement of peptidyl-tRNA from the acceptor (aminoacyl) site to the donor (peptidyl) site on reticulocyte ribosomes; the same effects were noted by these authors in yeast.

The experimental data presented in this thesis on the effects of cycloheximide indicate that derepression of FDPase after inactivation/repression by glucose requires de novo protein synthesis. The results further suggest that inactivation of FDPase by glucose is irreversible, and that reappearance of the enzyme is not simply activation of pre-existing molecules. Gancedo (1971) arrived at similar conclusions in his work with FDPase in S. cerevisiae and Ferguson et al. (1967) observed that cycloheximide prevented or diminished the induction (derepression) of malate dehydrogenase by acetate in the same organism.

Transcriptional control of inducible enzymes has been well documented for a variety of enzyme systems. More recent works, however, have indicated that in some cases, translation also seems to be affected by specific regulatory processes. In eucaryotic cells, post-transcriptional control over protein synthesis has been found in tyrosine amino transferase induction in rat hepatoma cells (Tomkins et al., 1969). These authors indicated that actinomycin D "superinduces" the synthesis of the amino transferase and that the effect may be due to a labile repressor of mRNA translation which was degraded in the presence of the antibiotic. Messenger RNA synthesis was the proposed regulated step in the derepression of the glucose uptake system in Neurospora crassa (Schneider and Wiley, 1971). These authors noted

that cycloheximide, although preventing derepression of the low Km glucose transport system, allowed mRNA for this system to accumulate by increasing the half-life of the message by blocking translation. Cybis and Weglenski (1972) worked with the induction of arginase in the fungus, Aspergillus nidulans. In their work, the transcription of mRNA for arginase was separated from mRNA translation by including cycloheximide addition with the inducer, arginine. These authors noted a 4.5-fold increased stability of mRNA under conditions of blocked translation and concluded that the latter phenomenon may account for mRNA accumulation in the presence of cycloheximide. That is, arginase specific mRNA is synthesized and stored in the absence of protein synthesis. In a study of tyrosinase induction in cultures of N. crassa, Horowitz et al. (1970) noted that actinomycin D and cycloheximide (both at high concentration) inhibited de novo synthesis of the enzyme during derepression by fasting. However, at low concentration, these antibiotics were seen to derepress the synthesis of tyrosinase. These authors assumed that enzyme synthesis is controlled by an unstable repressor protein; i.e., under low concentration of antibiotics, partial inhibition of protein synthesis occurs which lowers the repressor concentration thus freeing structural genes and allowing translation. Kynureninase induction in N. crassa in the presence of cycloheximide has been studied by Turner et al. (1970). These authors also were able to separate transcriptional events from translational control over enzyme synthesis. Rather than involving the presence of translational repressors, in the presence of cycloheximide and the inducer, kynurenine, mRNA specific for kynureninase accumulated in the absence of concomitant

translation.

The experiments outlined in the present investigation indicated that the initial "glucose effect" on FDPase in S. cerevisiae could not be prevented by actinomycin D. It also appeared that later effects of glucose (or one of its catabolites) on FDPase activity depend in part on protein synthesis and were sensitive to actinomycin D inhibition. Although the author does not invoke the induction by glucose catabolism of translational repressors, it does appear that repression by glucose of FDPase requires protein synthesis. Actinomycin D may be acting in inhibiting transcription of mRNA specific for FDPase and mRNA specific for those enzymes involved in the catabolism of glucose. Both effects would produce a decline in the level of FDPase. However, a release in actinomycin D inhibition would account for the rise in FDPase activity in the presence of glucose because previously synthesized FDPase-specific mRNA would now be translated more rapidly than induction of catabolite repressors by glucose. However, this interpretation implies that mRNA specific for FDPase is rather stable during inactivation/repression events.

Data which provide evidence that glucose inactivation/repression involves protein synthesis comes also from experiments with cycloheximide. The results indicated that in addition to preventing or diminishing glucose inactivation of FDPase, cycloheximide prevented those effects of glucose mediated through effects on protein synthesis. Data are asso presented to illustrate that cycloheximide, while preventing further mRNA translation, does not severely limit the expression of the existing enzyme. Even when the antibiotic was added 30 min after the

addition of repressive concentrations of glucose, no further declines in activity nor increases were noted. The specific activity of FDPase was frozen at the level present at the time of cycloheximide addition. These results are in part consistent with those of Ferguson et al. (1967) on glucose inactivation of malate dehydrogenase. However, the data are inconsistent with that given by Gancedo (1971) who found no effect of cycloheximide on inactivation of FDPase when the antibiotic was added together with 1.0% fructose.

The inactivation/repression of cellobiase by glucose has been described in the fungus, Chaetomium thermophile, by Lusis and Becker (1973). Cycloheximide was found to prevent induction by cellobiose which indicated that de novo protein synthesis of the enzyme was required; that reappearance of the enzyme after inactivation by glucose was not merely activation of existing enzyme molecules. The inactivation/repression of ornithine transcarbamylase (OTCase) in S. cerevisiae has been described by Bechet and Wiame (1965). In this work, cycloheximide, if added together with arginine, prevented the decrease in OTCase activity. The authors, however, assumed that inactivation involved the synthesis of a specific regulatory binding protein which acts directly on OTCase operating stoichiometric rather than catalytic resulting in loss of in vivo activity.

While studying the effects of various glucose concentrations on the inactivation of FDPase, this author noted that the higher the initial glucose concentration, the greater was the initial decline in activity of the enzyme. After the initial interval passed, and for the remainder of the experiments, a random series of repression-

derepression-like activities appeared. While no definitive conclusions can be drawn from these observations, it should be noted that the results of Jayaraman et al. (1966) are pertinent. In a study of respiratory enzymes during glucose repression, these authors noted an oscillatory pattern of repression-derepression. However, cytological evidence suggested that there was a decline of mitochondria and membrane-delimited mitochondrial profiles in the yeast cells during repression which reappeared during derepression in sequential steps. Further, Beck et al. (1968) proposed that possibly the effects of glucose repression may not be due to glucose (or its catabolites) but innately interlinked with the cell cycle events. In a study of seven respiratory-linked enzymes during growth on glucose and a shift to accumulated ethyl alcohol, these authors noted a correlation with a strong increase in the percentage of single (non-budding) yeast cells in the population and derepression of the majority of the enzymes. Hence, a correlation between the state of cells in the budding cycle and enzyme repression-derepression is suggested. While studying the oxygen uptake capacity of synchronously growing cultures of S. cerevisiae, Dharmalingam and Jayaraman (1973) noticed that oxygen uptake increased and decreased in an oscillatory pattern. When maltose was used as a carbon source, the oxygen uptake followed a stepwise increase in parallel with growth. Oscillatory patterns were seen only when glucose was the carbon source. Although mitochondriogenesis and respiratory capacities were not studied or measured in the present work, it appears that glucose-induced inactivation/repression of FDPase may well be related to the respiratory metabolism of the cells.

Since cycloheximide appeared to diminish inactivation of FDPase by glucose and inhibit repression of the enzyme, further attempts to prevent inactivation/repression by glucose by using inhibitors of energy-yielding metabolism were in general negative. One general exception was the observation that iodoacetamide prevented repression of FDPase by glucose. This agent also gave an identical response when added to cultures in the presence of glucose and sodium fluoride. Inactivation was still apparent but prolonged repression was no longer evident. This alkylating agent, iodoacetamide, is known to inhibit enzymes containing exposed (-SH) groups irreversibly in a fashion such that covalent derivatives of the enzymes are formed. Fluoride, on the other hand, during glucose catabolism in yeast should inhibit catabolic processes leading to the accumulation of phosphate esters like 3-phosphoglycerate. This, in affect, may explain why the enzyme level stabilizes during the latter stages of exposure to glucose and sodium fluoride. However, this also implies that the catabolite of glucose responsible for repression of FDPase may be produced in the glycolytic scheme before the formation of 3-PGA. Later experiments revealed that 3-PGA does not engage in inactivation/repression but appeared to be slightly inductive in its response. Iodoacetamide should inhibit the action of at least one enzyme, glyceraldehyde 3-phosphate dehydrogenase, leading to the accumulation in yeast cells of fructose 1,6-diphosphate during glucose catabolism. Since fructose 1,6-diphosphate is the normal substrate of FDPase, this effect of iodoacetamide in producing a non-repressive response of glucose may be thusly visualized.

In addition, although 5-methyl tryptophane did not prevent

inactivation of FDPase by glucose, it interfered with the repressive response of the added sugar. Further, fructose and mannose also elicited inactivating effects on FDPase. These observations imply that glucose itself is not the catabolite responsible for inactivation/repression of FDPase. In toto, the inescapable conclusions at this juncture are that inactivation/repression of FDPase by glucose (or its catabolite/s) involves initially direct enzyme inactivation with prolonged effects depending upon in part and affecting de novo protein synthesis.

Observations presented further reveal that an analogue of glucose, 2-deoxy-D-glucose, caused inactivation of FDPase although not as extensive as glucose itself. This observation, along with the effects of iodoacetamide on the "glucose effect" are not consistent with the data reported by Gancedo (1971). He reported that 2-deoxy-D-glucose did not cause inactivation of FDPase in S. cerevisiae nor did iodoacetamide affect glucose inactivation. The author's data on the effects of 2-deoxy-D-glucose on FDPase activity are, however, consistent with the data reported by Witt et al. (1966) with malate dehydrogenase. Similar results with MDH were obtained by Ferguson et al. (1967), however, the latter author concluded that 2-deoxy-D-glucose, by behaving as a general metabolic inhibitor of protein synthesis, does not repress MDH in the classical sense but prevents enzyme induction. It should be stated that 2-deoxy-D-glucose is not significantly catabolized in yeast beyond 2-deoxy-D-glucose 6-phosphate (Witt et al., 1966). However, Tyler and Magasanik (1970) noted in E. coli that "transient repression" of beta-galactosidase occurs in the presence of 2-deoxy-D-glucose after

growth in another carbon source. Further, in their work, L-alpha-glycerophosphate elicited transient repression in the absence of L-alpha-glycerophosphate dehydrogenase. These results were taken as evidence that newly added compounds need not be catabolized in order to repress. Tyler and Magasanik (1970) further suggested that the actual passage of the compound through the cell membrane is responsible for the repression.

It is suggested in the present work that the response of FDPase to 2-deoxy-D-glucose may well represent transient repression rather than catabolite repression. Further, it is inferred that the initial inactivating effects of glucose on FDPase activity may be due in part to transient repression with the cycloheximide and actinomycin D-sensitive effects of glucose due to catabolite repression.

The data from experiments dealing with the effects of nitrogen starvation on glucose-induced inactivation/repression of FDPase are not easily interpretable. Until further experimentation can be performed, tentatively, inactivation appears to occur even in cells incubated without a nitrogen source. A more dramatic type of inactivation is produced, however, during prolonged exposure to glucose without nitrogen and the oscillatory behavior of FDPase in the presence of glucose is diminished. If one further assumes that the glucose permease system is present during induction with acetate, then subsequent inactivation of FDPase by glucose would be independent of de novo protein synthesis and not require a nitrogen source. Further, transient repression would be operative upon glucose addition and this phenomenon would, in addition, account for a greater inactivating response in the

absence of nitrogen. The data illustrating the expression of FDPase after 4 hr nitrogen starvation suggest that mRNA specific for FDPase can be stored during nitrogen starvation, remains stable during glucose inactivation and can be later translated into FDPase. These conclusions are tentative and further experimentation is deemed necessary.

From experiments designed to elucidate the effects of phosphorylated sugars on the induced level of FDPase, results indicated that only glucose 6-phosphate had any measurable repressive effects. However, the results also indicate that there was negligible growth in this carbon source. It should be kept in mind that a type of transient repression may be involved and that the experiments of Tyler and Magasanik (1970) have established that a newly added compound need not be catabolized in order to repress enzyme synthesis.

The experiments of Faith et al. (1964) tend to exclude glucose 6-phosphate as the catabolite of glucose involved in the repression of induced beta-galactosidase formation in E. coli. These authors measured the glucose 6-phosphate concentration in nitrogen starved cells and found that the resumption of induced beta-galactosidase formation coincided with the disappearance of exogenous glucose. These events occurred although the intracellular concentration of glucose 6-phosphate remained high.

It should be noted in the present work that the repressive response on FDPase elicited by glucose 6-phosphate does not approach the inactivating effects of glucose. However, the data do not exclude glucose 6-phosphate as a contributory catabolite in glucose-induced inactivation/repression of FDPase activity.

CHAPTER VI

SUMMARY

1. Fructose 1,6-diphosphatase (FDPase) from a derepressed (acetate or pyruvate-induced) culture of S. cerevisiae was found to undergo rapid "inactivation/repression" after the addition of glucose to the culture medium.

2. Experiments with actinomycin D have indicated that de novo protein synthesis is required for the reappearance of the enzyme during derepression (induction).

3. A time-dependent release of actinomycin D inhibition of derepression of FDPase was noted. Cells of S. cerevisiae recover the capacity to synthesize the enzyme.

4. Cycloheximide prevented a rise in measurable activity of FDPase during induction (derepression) by acetate.

5. Inactivation of FDPase by glucose (or its catabolites) could not be prevented by actinomycin D. However, prolonged "glucose effects" were sensitive to inhibition by the antibiotic.

6. Inactivation of FDPase by glucose could not be prevented by cycloheximide; however, prolonged catabolite repression of the enzyme activity was inhibited by the antibiotic.

7. Results from experiments using antibiotics known to inhibit protein synthesis at the transcriptive and translational levels indicate that in the presence of glucose, initially, FDPase undergoes inactivation and a type of transient repression probably ensues. However, continued repressive effects of glucose on FDPase depend upon and

affect protein synthesis.

8. Correlations between the initial glucose concentrations in the culture media and the extent of glucose-induced inactivation/repression of FDPase were not evident.

9. Several metabolic inhibitors were tested and showed no apparent effect on glucose inactivation/repression with the exception of iodoacetamide.

10. Fructose, mannose and an analogue of glucose, 2-deoxy-D-glucose, were found to cause inactivation of FDPase. These data were taken as evidence that glucose itself is not the metabolite responsible for inactivation/repression of FDPase.

11. Nitrogen starvation of yeast cultures does not prevent the "glucose effect". On the contrary, amplification of the inactivation/repression response was noted.

12. The effects of phosphorylated sugars on the induced level of FDPase indicated that only glucose 6-phosphate had any measurable repressive effects. However, negligible growth occurs in the presence of this carbon source.

13. Results from all experiments lead to the conclusions that glucose (or its catabolites) initially causes direct enzyme inactivation of FDPase after induction on a non-fermentable carbon source. Further, a type of transient repression is probably involved in the "glucose effect". However, prolonged effects of inactivation/repression depend upon and affect protein synthesis.

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